University of Alberta

Slow-scalding in barley

by

Behzad Sorkhilalehloo ©

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To my wife,

Nasrin

and to our son,

Amyn

Thank you for your warm support, kind consideration, and unconditional love!

Abstract

Slow-scalding resistance (S-SR), the phenomenon of slow disease development under severe epidemic of scald caused by *Rhynchosporium secalis* (Oud.) J. J. Davis, has been gaining increasing attention in barley improvement due to concerns regarding the instability of major-gene resistance.

Indoor and field techniques were optimized to ensure consistently uniform development of infection on barely genotypes at both the seedling and adult plant stages.

Using the optimized techniques, the quantitative reaction of 38 western Canadian barley cultivars to mixtures of scald isolates under different field conditions in Canada and Mexico were determined during 1999-2001. Slow-scalding genotypes were defined and identified by the presence of susceptible reactions at the seedling stage and a low to intermediate level of disease progress at the adult plant stage.

Large pathogenic variability was observed among 19 single-spore isolates of *R. secalis* studied in pathogenicity tests using a set of 73 barley genotypes. It was hypothesized that genotypes with resistance against a higher number of pathogen isolates could perform in a more durable/stable way in the field.

Histological studies revealed that in the slow-scalding cultivars, rates of sporulation were lower compared to those in susceptible genotypes. The changes in levels of calcium, potassium, silicon, and sodium in the superficial layers of leaves suggested the involvement of active mobilization of and/or sequestration of elements in this pathosystem. It was found that severe auricle infections may adversely affect transport of photosynthates from the leaf into the plant.

The genetic basis of S-SR was investigated in several Canadian and ICARDA/CIMMYT derived genetic populations both in Canada (Alberta) and at Toluca (Mexico). The results revealed the existence of 1-4 resistance genes with additive effects and high heritabilities in the slow-scalding parental lines. Reactions and cross-location resistance stability of susceptible, resistant, and S-SR genotypes and segregating populations varied significantly between Mexican and Canadian locations. These differences were attributed to major pathogen differences in the two countries, which were able to reciprocally overcome S-SR expressed in each country. It was concluded that achieving more stable genetic resistance to scald through breeding for S-SR is both practical and feasible.

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List of symbols and abbreviations

AFLP	Amplified fragment length polymorphism
AI	SAUDPC for %I
AS	SAUDPC for %S
BSA	Bulk(ed) segregant analysis
CIMMYT	International wheat and maize improvement center
cv.	Cultivar
DH	CDC Dolly 6 x RFLP Harrington population
DH lines	Doubled haploid lines derived from the cross CDC Dolly x TR 251
DI	Disease index
DMP	Deviation from mid-parents
DT	CDC Dolly x TR251 population
FI	Final %I
FS	Final %S
GCA	General combining ability
G x E	Genotype by environment interaction
h	Hour
H	Heritability
HRL	Hypersensitive response-like
HSD	Tukey's honestly significant difference
I	Incidence
ICARDA	International center for agricultural research in the dry areas
ITS	Internal transcribed spacer
km	Kilometre
L	Litre
LBA	Lima bean agar
LM	Light microscopy
m	Meter
MAS	Marker assisted selection
min.	Minute
MP	Mid-parent values
PCR	Polymerase chain reaction
PR	Pathogenicity related
QTL	Quantitative trait loci
r	Apparent infection rate/ Coefficient of correlation
RAPD	Random amplified polymorphic DNA
RCBD	Randomized complete block design
RFLP	Restriction fragment length polymorphism
rI	r for %I
RIL	Recombinant inbred line
rS	r for %S
RxR	Resistance intercrosses
S	Severity
S-SR	Slow-scalding resistance
SAUDPC	Standardized area under the disease progress curve

SCA	Specific combining ability
SE	Standard error
sec	Second
SEM	Scanning electron microscopy
SKW	Skewness
SSD	Single-seed descent
SSR	Simple sequence repeat (microsatellite)
Std, std	Standard deviation
STS	Sequence tagged site
SxR	Susceptible x resistant crosses
TEM	Transmission electron microscopy
TS	Terminal severity
wk	Week
XRM	X-ray microanalysis
ZGS	Zadoks' growth stage
χ^2	Chi square
σ^2_{gca}	General combining ability variance
σ^2_{r}	Reciprocal variance
σ^2_{sca}	Specific combining ability variance

Chapter 1

Barley-scald pathosystem: Literature review

1.1. Introduction

Globally, barley is the fourth most important crop today, playing a prominent role in animal feed, malting, and human food industries. Although, its major use is as animal feed, demand for high quality malting barley has increased recently. Its ability to adapt to a broad range of environmental conditions and climates has enabled barley to produce economic yields in areas where other crop species cannot (Chopra and Prakash, 2002). Barley is grown over 52 million hectares of agricultural areas around the globe with a total annual production of over 132 million metric tons (FAO, 2003). Canada was the seventh largest producer of barley in the world in 2002 (FAO, 2003). Most of the barley produced in Canada comes from the prairies, within which Alberta plays a major role (McLelland, 1989). According to Statistics Canada, 2001, there were about 2,206 million hectares of barley in Alberta (Anonymous, 2002). This was 44% of the total Canadian acreage and 47% of the acreage in western Canada (Cortez *et al.*, 2002).

Crops can suffer severe losses in yield and quality as a result of plant diseases (Agrios, 1997). It has been estimated that about 30% of the world crop production is lost every year and one-third of these losses are due to plant diseases (Fraser, 1985). Barley in North America is affected by about 15 economically important diseases (Kiesling, 1985). Scald or leaf blotch of barley, caused by *Rhynchosporium secalis* (Ayres) Davis, is one of the most important fungal diseases of barley in cool and semi-humid areas throughout the world and can cause yield losses of 35% or more (Shipton *et al.*, 1974). In western Canada, the incidence of scald disease has increased since the early 1950's as a consequence of improper use of cultural practices and the widespread use of susceptible varieties (Skoropad, 1960; 1976; Xue *et al.*, 1994). Yield losses of up to 30% have been reported in Alberta (Skoropad, 1960; Xue *et al.*, 1994). None of the registered malting barley cultivars currently grown in western Canada are resistant to scald (Xue and Hall, 1991). Consequently, breeding for scald resistance is a prime objective in the barley breeding programs in Alberta.

The objective of this chapter is to provide literature review on the barley-scald pathosystem relating to all topics investigated in this thesis.

1.2. Barley

1.2.1. History and origin

Since 18,000 BC, the history of mankind has also been the history of barley which is one of the first plant species to be used as food and most probably the first one to be domesticated as a crop (Nicola *et al.*, 2002). Domestication of barley probably started with its wild relative *Hordeum spontaneum* C. Koch. in the Fertile Crescent about 10,000 years ago (Bothmer and Jacobson, 1985).

1.2.2. Taxonomy and related species

The genus *Hordeum*, belongs to *Triticeae* tribe of the family *Poaceae* (Bennett, 2000). *Hordeum spontaneum* or *H. vulgare* L. ssp. *spontaneum*, the wild ancestor of the cultivated barley *H. vulgare*, is two-rowed, annual, self-pollinated, diploid ($2n = 2x = 14$), and brittle (Pickering and Devaux, 1992). Among different species belonging to this genus, *H. spontaneum* is cross-compatible with the cultivated barley and has been considered as a valuable source of useful resistance genes to abiotic and biotic stresses in breeding programs (Bothmer, 1992; Ellis, 2002). Various disease resistance genes have been detected in this species (Abbott *et al.*, 1992; Czembor and Gacek, 1987; Moseman *et al.*, 1983). A perennial species, *H. bulbosum* L., is closely related to cultivated barley, but possesses sterility inducing factors (Pickering, 1988; Pickering and Devaux, 1992). It has been used in breeding to achieve *H. vulgare* doubled haploids. The perfect chromosome pairing between the two usually results in the elimination of *H. bulbosum*'s genome (Anderson and Reinbergs, 1985; Poehlman and Sleper, 1995). All other species of the genus *Hordeum* are potential sources of useful genes, though their hybrids are usually sterile, and can be used for hybrid crosses via embryo rescue and other *in vitro* fertilization techniques (Jörgensen, 1992; Pickering, 1988; Walther *et al.*, 2000). Relationships among various species of *Hordeum* at the genomic level have delineated four basic groups: *vulgare-bulbosum*, *marinum*, *murinum*, plus one other species from

Asia and America (Baum and Bailey, 1991; Bothmer and Jacobson, 1985; Monlar *et al.*, 1992).

1.3. Barley scald disease

Scald of barley is an economically important disease of barley worldwide. Its importance has increased in modern farming systems especially due to continuous cultivation of barley and use of minimum tillage practices (Bailey, 2002; Yahyaoui, 2002). The disease reduces both barley yield and quality, and may cause multi-million dollar losses annually (Anonymous, 1999). The causal agent of the disease, *Rhynchosporium secalis* (Oud.) J. J. Davis (syn. *Marsonia secalis* Oudem.), is a fungus that exhibits a degree of specialization with regard to its host range (Mathre, 1997). It occurs on rye, triticale, and certain grass species, including *H. leoporinum* Link, *H. murinum* L., *H. glaucum* Steud., and *Elymus triticoides* Buckl. (Albertini, 1995; Jarosz and Burdon, 1996; Mathre, 1997). In the United States alone, the fungus occurs on *Agropyron, Agrostis, Bouteloua, Bromus, Dactylis, Danthonia, Elymus, Hordeum, Leymus, Lolium, Phalaris, Phleum, Poa, Quercus,* and Secale (Farr *et al.*, 1995). Shipton *et al.* (1974) have published a comprehensive review of literature on the barley scald disease. The current review is designed to provide an update of developments on barley scald since the previous literature survey was completed in 1974.

1.3.1. Causal organism and symptoms

Rhynchosporium secalis is the imperfect state of the fungus that causes scald. No sexual state is known for this fungus. The mycelium of *R. secalis* is hyaline to light gray and develops as a compact stroma under the cuticle of the host plant. Conidia are one-septate, hyaline, and cylindrical to ovate, and most have a short apical beak. They are 2-4 x 12-20 µm in size and are borne on cells of the stroma. Microconidia of the pathogen have been reported to exude from flask-like phialides (Skoropad and Grinchenko, 1957), but their function has yet not been elucidated (Mathre, 1997). As shown in Figure 1-1, the distinctive lesions of scald occur on coleoptiles, leaves, leaf sheaths, glumes, floral bracts, and awns (Shipton *et al.*, 1974). Young lesions are oval to irregular blotches with a dark or pale gray or blue-gray color. They may look water-soaked later. As the infection advances, the centers of lesions dry out and turn light gray to white, and their edges turn dark brown and may be surrounded by a chlorotic area (Mathre, 1997). Lesions are not delimited by the veins and may have a zonate appearance. In severe epidemics, the entire leaf may be destroyed by the pathogen. During artificial inoculation, the seedlings of susceptible barleys usually show typical scald symptoms. However they may wilt completely about 2 weeks after inoculation (Mathre, 1997). This might be due to a very high concentration of inoculum and consistent wet conditions. As an unusual symptom, some Ethiopian barley cultivars inoculated with an isolate of scald from Montana have shown no lesions on their leaf blades, but brown discoloration and subsequent wilting of the leaf sheaths (Nyvall, 1989). Symptomless leaf-infection by the scald pathogen has been also reported for some resistant cultivars (Ali, 1974). Symptomless seed-infection by *R. secalis* can be identified by immunofluorescent detection (Mille, 1990), and both by cultural and polymerase chain reaction (PCR)-based detection assays (Lee *et al.*, 2001a).

1.3.2. Taxonomy and phylogeny

Rhynchosporium secalis is a haploid fungus that belongs to the kingdom Fungi and the phylum Ascomycota. This fungus is placed within the anamorphic group Deuteromycetes (Agrios, 1997; Goodwin, 2002). Due to a paucity of morphological features, classification of *R. secalis* has been difficult. As reported by Goodwin (2002), the genus *Rhynchosporium* contains three species i.e. *R. secalis*, *R. orthosporum* R. M. Caldwell and *R. alismatis* (Oudem) J.J. Davis. Conidia of *R. secalis* typically are produced on mycelia directly with no true conidiophores (Caldwell, 1937). *Rhynchosporium orthosporum* causes scald or white leaf stripe of Poaceae. It is very similar to *R. secalis* morphologically, but its conidia are uniformly cylindrical. The main host of *R. orthosporum* is orchardgrass (*Dactylis glomerata* L.), however it has been observed in several other host species of the genera *Agropyron*, *Agrostis*, *Alopecurus*, *Calamagrostis*, *Elymus*, *Festus*, *Lolium*, and *Poa*, which overlap with the host range of *R. secalis* (Farr *et al.*, 1995). In contrast with the aforesaid species, a third species, *R. alismatis*, can be excluded from the *Rhynchosporium* genus because it produces conidia from conidiophores and lacks a superficial stroma (Caldwell, 1937). *Rhynchosporium alismatis* is known to infect many hosts in the family *Alismataceae* and is being developed as a potential biocontrol agent for the weedy members of that family (Cother, 1999).

Until recently, little was known about the phylogenetic relationships of *R. secalis*. Internal transcribed spacer (ITS) regions have been used for developing species-specific probes or for designing primers for detection of *R. secalis* from other fungi including *R. orthosporum* (Lee *et al.*, 2001b; Lee, 2002). Goodwin *et al.* (2002) have studied the relationship between *R. secalis* and other fungi through phylogenetic analysis of the 18S ribosomal RNA gene and the ITS region. The results indicated that 18S sequences were not useful in elucidating the phylogenetic relationships of *R. secalis*. However, analyses of 76 ITS sequences revealed very close relationships among the anamorphic ascomycete *R. secalis* and species of the teleomorphic discomycete genera *Tapesia* and *Pyrenopeziza*. In addition to *R. secalis*, this monophyletic group also included *R. orthosporum*. Obviously, much additional work is needed to clarify the taxonomic relationships of *Tapesia*, *Pyrenopeziza* and other species related to these taxa. On the basis of these results, the teleomorph of *R. secalis*, if it exists, most likely will be a species of *Tapesia* with a small apothecium produced directly on dead, infested host tissue (Goodwin, 2002). Formerly, Salamati *et al.* (2000) studied several populations of *R. secalis* from Norway, Finland, and Australia, and postulated that *R. secalis* must have an unrecognized telemorph. It was also suggested that the studied population underwent regular recombination that could be an indication of sexual reproduction in the fungus (Salamati *et al.*, 2000). In *R. secalis*, microconidia are sometimes formed in conjunction with normal conidial formation (Skoropad and Grinchenko, 1957). In some Ascomycetes, microconidia act as spermatia or male gametes (Alexopoulos *et al.*, 1996). Microconidia of *R. secalis* do not germinate and their function as sexual gametes has not been proven (Ali, 1972).

1.3.3. Pathogenic variability

Despite the absence of a known telemorph, a wide and high level of pathogenic variation has been reported for many studied *R. secalis* populations in different regions of the world (Goodwin *et al.*, 1990; Habgood, 1973; Jackson and Webster, 1976; Jörgensen and Smedegaard, 1995; McDermott *et al.*, 1989; McDonald *et al.*, 1999; Meles *et al.*, 2004; Robbertse and Crous, 2000; Salamati and Tronsmo, 1997; Tekauz, 1991; Xue *et al.*, 1991). The results have been confirmed using several different genetic markers including isozymes, ribosomal DNA, and RFLP (Goodwin *et al.*, 1994; Goodwin *et al.*, 1993; McDermott *et al.*, 1989; McDonald *et al.*, 1999; McDonald *et al.*, 1989). Numerous pathogenic races or pathotypes have been identified on the basis of the reactions of differential barley cultivars or lines (Goodwin *et al.*, 1990; Tekauz, 1995). Interestingly, single spore isolates of the pathogen from different lesions of the same plant and even from the same lesion have been shown to be significantly different in their sporulation rate, pathogenicity, and genetic marker banding patterns (Brown, 1985b; Brown, 1990; Habgood, 1973). In contrast, because the fungal spores are primarily dispersed by rain splash (Shipton *et al.*, 1974), the gene flow between *R. secalis* populations is reported to be limited (Tekauz, 1995). Other reports on several different genetic populations of the scald pathogen indicate that *R. secalis* must have a medium effective population size and medium gene/genotype flow (McDonald and Linde, 2002; Salamati *et al.*, 2000). Knowledge of local, regional, and global genetic variation of *R. secalis*, and virulence of the pathogen populations is highly important for planning disease management and for breeding durable scald resistance (Xi *et al.*, 2002; Xi *et al.*, 2003). Jarosz and Burdon (1996) have mentioned that the existence of widespread resistance variation in wild barley grasses (i.e., *Hordeum glaucum* and *H. leporinum*) may create substantial selection pressures suitable for the emergence of variability for pathogenicity in the fungus which would complicate efforts to breed for scald resistance in cultivated barley. Fukuyama *et al.* (1998) investigated variation of virulence in *R. secalis* distributed in the Hokuriku district of Japan. Using 14 differential barley cultivars and a total of 38 cultures isolated from the infected cultivar Minorimugi, a complex variation in the virulence pattern was observed and a geographical cline for virulence was observed. It was suggested that the virulence of the fungus is conditioned not only by race-specific gene(s), but also by some genes with interactions such as additive or complementary effects.

On the other hand, several studies have been undertaken to elucidate the possible sources of variation among the pathogen isolates (Burdon *et al.*, 1994; Goodwin *et al.*, 1994; Goodwin *et al.*, 1993; Konovalova, 2001a; b; McDermott *et al.*, 1989; McDonald *et al.*, 1999; Newman and Owen, 1985; Newton, 1989). Spontaneous mutation and migration, parasexuality, and unobserved sexual reproduction coupled with the presence of alternative wild host plants (Brown 1990) have been hypothesized to be of the most important possible factors in generating the observed extensive genetic variation among and within different populations of the pathogen, and exerting sustained selection pressure for greater pathogen virulence (Brown, 1990; Goodwin *et al.*, 1994; Konovalova, 2001b; Salamati *et al.*, 2000). In contrast, McDermott *et al.* (1989) studied genetic variability for pathogenicity, isozyme, ribosomal DNA, and colony color variants in populations of *R. secalis* and found that negligible recombination occurred in the populations studied.

Studies on the barley-scald pathosystem have long been plagued by a set of problematical issues, including the lack of universally-accepted differential sets for both barley and the scald pathogen (Wallwork, 2002). Using a standard differential set, plant breeders and plant pathologist can study both pathogen populations and assess desirable resistant germplasm for breeding programs. However, it may not be a guarantee that comparisons between the disease populations will be meaningful. As Tekauz (1995) describes, there are at least five factors that make interpretation difficult, and influence the repeatability of obtained results. The factors are (1) the access of researchers to different seed sources, which may result in using genetically variable genotypes; (2) differences in aggressiveness of races between regions, which affect the severity and incidence of the disease; (3) the inoculation procedures and conditions used, which cause different levels of epidemics; (4) the age and status of the cultures; and (5) the scoring system, which is one of the main obstacles in obtaining consistent results. In turn, using one source of a standardized differential set, preferably after general agreement between plant breeders and pathologists, would alleviate the first problem. Standardization of factors affecting the optimum growth of barley scald and maximum expression of disease would also help to maintain uniformity between different experiments. The problem with scoring systems is also considered to present a real difficulty in proper decision making and screening of breeding materials (Williams and Owen, 1973). Quantitative studies on the genetics of resistance require a numerical system to quantify disease reactions. The 0-9 scoring system (Couture, 1980) is currently used by some scald workers to score field reaction of barley genotypes to mixtures of natural and artificial *R. secalis* isolates. However, in many instances, there is no agreement between the defined/expected percentage leaf area scalded in intermediate scores of this scale and what is observed in the field condition. For example, a plant with scattered lesions on the upper leaves and 5-10 percent scalded area on the lower leaves does not fit into this system. At the International Maize and Wheat Improvement Center (Centro Internacional de Mejoramiento de Maíz y Trigo, CIMMYT), a qualitative disease rating system is used to screen the breeding materials resistant to *R. secalis*, and to determine the sources of resistance (Ginkel and Vivar, 1986). However, to compare individuals scored as intermediate, moderately resistant or moderately susceptible in different rating systems, a well-defined scale is needed to cover all aspects of disease progress. With respect to the pathogen, Goodwin *et al.* (1990) addressed the difficulty of comparing results of separate studies and proposed a new system of nomenclature, which can be used to convert the previously identified and non-uniformly named pathotypes in to a common nomenclature.

1.3.4. Epidemiology

Rhynchosporium secalis is widespread in Europe and North America (Mathre, 1997). It has also been reported in Africa, Australia, Korea, Japan, the Middle East, and New Zealand (Brown, 1985a; Burdon *et al.*, 1994; Cromey, 1987; Golzar, 1995; Mathre, 1997; Robbertse *et al.*, 2000).

The scald fungus overwinters on the seed and stubble of diseased barley crops (Shipton *et al.*, 1974). It may also overwinter as stroma in lesions on winter barley (or any other host species) infected during the fall (Ayesu Offei and Carter, 1971). The fungus can survive much longer in crop residue that remains above the soil surface rather than on the surface or in the soil (Mathre, 1997). It cannot survive saprophytically in the soil (Mayfield and Clare, 1984). It has been reported that under field conditions, the fungus can sporulate for up to 340 days (Skoropad, 1965). Under laboratory conditions, diseased leaves may produce spores for about 16 months and a low amount of sporulation can be expected even after 24 months (Shipton *et al.*, 1974).

As shown in Figure 1.2, in spring and other times when cool and humid weather prevails, healthy barley plants are primarily infected by conidia produced on the host residues, which are the principal source of primary inoculum. Infected seed can be another source of primary inoculum since *R. secalis* can also survive as mycelium in the pericarp and the hull of barley seeds where the coleoptile can be infected as it emerges from the embryo at a soil temperature of 16°C (Skoropad, 1959). Natural openings at the tips of coleoptiles can also trap conidia and provide conditions for their germination that may lead to further seedling infection (Skoropad, 1959). Although, barley can be attacked at any stage of its growth, the disease is usually most severe just before and during the heading stage (Shipton *et al.*, 1974). The disease is also more severe when the crop is grown over winter in areas with winter rainfall (Brown *et al.*, 1996). Conidia production is optimal between 15 and 18°C, and within 72 hours of wetting (Anonymous, 2003). Wetting periods longer than optimal lead to death of spores (Shipton *et al.*, 1974). The optimal temperature for spore production is also favorable for the whole infection process (Caldwell, 1937). A 24-hour, moist period is sufficient for spores to infect leaves. Following the moist period, lesions appear in 12-24 days when plants are kept at 20°C and 40-60% relative humidity for 12 hours per day, alternating with 12 hr of darkness at 15°C (Davis and Fitt, 1994; Mathre, 1997). The fungus sporulates only on wet lesions after the leaf tissue has become necrotic and lesions reach their maximum development in two weeks (Caldwell, 1937). Usually, a disease cycle may take two weeks from the early stage of conidia germination to the formation of mature spores on infected barley tissues (Skoropad, 1959). Studies on meteorological factors influencing the barley scald disease have revealed that disease incidence was influenced strongly by both the precipitation frequency and the total degree days resulting from the daily accumulation of differences between 10°C and minimum temperatures lower than 10°C (Carmona *et al.*, 1997). Severity of disease in the previous crop and the environment to which the residue is exposed control the amount of primary inoculum (Shipton *et al.*, 1974). Secondary inoculum is produced from new lesions on the surface of lower leaves (Mathre, 1997). The amount of primary inoculum, the number of cycles of secondary infections, host susceptibility, and environmental conditions are the most important factors in development of scald epidemics (Anonymous, 2003; Davis and Fitt, 1992; Mathre, 1997). Temperatures from 27-37°C retard sporulation and greatly restrict secondary infections (Skoropad, 1965). Ayesu-Offei and Carter (1971) reported that sporulation occurs when free water is available. Their observations support that release and dispersal of the conidia are mainly the result of water splash due to rain or overhead irrigation. However, splashed spores may disseminate a short distance by wind to other barley plants. Because there is no significant long-distance conidium dispersal, disease development depends mainly on local inoculum (Salamati *et al.*, 2000). Long distance dispersal of the pathogen occurs through transportation of infected seed and straw by humans (Habgood, 1973). It has also been hypothesized that ascospores from an unknown teleomorph may be disseminated over distances of up to hundreds of kilometers (Salamati *et al.*, 2000).

1.4. Barley-scald interaction

Studies on several different host-pathogen pathosystems have revealed considerable information regarding the host-pathogen interactions (Crandall, 1987; Cselenyi and Friedt, 1998; Doken, 1988; Elen, 1986; Magnus, 1980; McDonald *et al.*, 1989; Van der Plank, 1982). The interaction between barley and the scald pathogen have been studied in various ways-microscopically, genetically, and biochemically. Several studies have been carried out to characterize the infection process and chemical elements/compounds involved in the pathogenesis of *R. secalis* (Gierlich *et al.*, 1999a; Gierlich *et al.*, 1999b; Gilgenberg Hartung, 1999; Hahn *et al.*, 1993a; Jörgensen *et al.*, 1993b; Mazars *et al.*, 1989a; Mazars *et al.*, 1990; Tewari, 2000; Turkington *et al.*, 1999; Xi *et al.*, 2000b). Histological examinations of the compatible interaction between *R. secalis* and susceptible barley lines have revealed many facts with respect to the infection process of the pathogen (Ayesu Offei and Clare, 1970; Caldwell, 1937; Doken, 1988; Jones and Ayres, 1974). Also, studies on the infection processes of compatible in comparison with incompatible interactions between *R. secalis* and susceptible versus resistant genotypes of barley have been carried out to investigate possible resistance mechanisms of the host (Jörgensen *et al.*, 1993a; Xi *et al.*, 2000b). As depicted in Figure 1.3, in a susceptible reaction, upon physical contact of conidia with the host tissue, conidial cells germinate and form appressoria prior to cuticular penetration. Penetrated mycelium then develops into stromata and invades epidermal cells (Caldwell, 1937). The pathogen does not produce any haustoria (Jörgensen *et al.*, 1993b). The mesophyll and vascular tissues may also be invaded at a later stage (Doken, 1988). Sporulation, development of conidia on short conidiophores, occurs on the otherwise intact cuticle. In the resistance reaction, in turn, appressorial formation and attempted cuticular penetration seem to be similar to those observed in the susceptible interaction. However, inhibition of fungal penetration has been found to be significantly correlated with papilla size. The papilla and haloes in the cell walls around the penetration pegs are larger in the resistant hosts compared to those of susceptible genotypes (Jörgensen *et al.*, 1993b). Prevention of attempted cuticle penetration by production of chemical barriers was suggested as another possible mechanism of resistance in the barley-scald pathosystem (Xi *et al.*, 2000b).

Different phytotoxic metabolites of *R. secalis* have been found (Gierlich *et al.*, 1999a; Gilgenberg Hartung, 1999; Hahn *et al.*, 1993c; Kilby and Robinson, 2001; Roulin *et al.*, 1997; Wevelsiep *et al.*, 1991; Wevelsiep *et al.*, 1993). It was shown that a low concentration of the phytotoxic glycoprotein toxin can induce chlorotic and necrotic symptoms similar to those observed in the last stages of the disease on barley leaves (Mazars *et al.*, 1989b). Collapse of epidermal walls, tissue necroses, and xylem-vessel plugging were also observed as the cell modifications induced by the phytotoxic glycoprotein (Auriol *et al.*, 1978). Biosynthesis of pathogenicity-related (PR) proteins has been considered to be a plant resistance response in some barley genotypes (Hahn *et al.*, 1993b; Roulin *et al.*, 1997). Antifungal proteins found in the intercellular washing fluid (IWF) have also been shown to play a possible role in defence against the pathogen (Zareie *et al.*, 2002). Roulin *et al.* (1997) found that the increased (1→3)-β-glucanase activity was attributable predominantly to a 2-3 day period in which mRNA transcripts of genes that encode isoenzymes GII and GI accumulated. They suggested that these genes may play an important role in the defense mechanism of the scald-barley pathosystem and they concluded that at least one physiological mode of resistance to the *R. secalis* is accompanied by (1-->3) - β- glucanase induction. The *R. secalis*-barley interaction provided the first example of a pathosystem conforming to the gene-for-gene hypothesis in which barley genotypes with Rrs1 resistance gene recognizes the pathogen by its avirulence gene, avrRrs1. With respect to the pathogen, recognition by the host plant was reported to be eluded by either deletion of the encoding gene or alteration of the primary structure of the gene product (Rohe *et al.*, 1995). NIP1, a small phytotoxic cysteine-rich protein secreted by the barley pathogen *R. secalis*, is a race-specific elicitor of defense responses (essential for the specific recognition of the fungus expressing avrRrs1 gene) in barley cultivars carrying the resistance gene, Rrs1 (Gierlich *et al.*, 1999a). As part of the barley defense response, significant accumulation of PRHv-1, a thaumatin-like PR protein, and peroxidase transcripts was induced early during pathogenesis in cultivars having Rrs1 resistance gene but not in the susceptible rrs1 cultivar or a cultivar lacking known resistance genes (Hahn *et al.*, 1993a). Therefore, NIP1, a candidate for the product of fungal avirulence gene avrRrs1, together with the resistance gene Rrs1, determine the incompatibility of interaction in this system. Rhynchosporoside, a high molecular weight phytotoxic glycoprotein secreted by *R. secalis* was found to be associated with the host membrane protein receptor (Mazars *et al.*, 1983). This glycoprotein was thought to be the possible reason for leaf chlorosis and necrosis due to plugging of the xylem vessels (Auriol *et al.*, 1978). It was suggested that rhynchosporosides are minor determinants of the pathogenesis induced by *R. secalis* (Branchard, 1984). The interactions between barley (*H. vulgare*) and two necrotrophic pathogens, *R. secalis* and *Pyrenophora teres* Drechs. (the causal agent of barley net blotch disease) have been investigated in another study (Able, 2003). The result showed that reactive oxygen species (ROS) may play a role in the induction of cell death in the susceptible plants during the challenge with both fungi. In two to three epidermal cells adjacent to phenolic browning and cell death, ROS was detected during the susceptible response. Interestingly, in the barley-scald interaction ROS, superoxide ($HO_2^{\cdot}/O_2^{-}$) was also found at a significant level in resistance responses. In other pathosystems, ROS has been reported to play a major role in initiation of hypersensitive response, which is a localized mechanism of resistance against obligate parasites. Furthermore, ROS serves to signal to other cells in the plant, inducing systematic acquired resistance, SAR (Heath, 2000). Although these kinds of reactions have not been reported in the barley-scald pathosystem, the study of ROS may lead to an understanding of other possible resistance mechanisms involved in the resistance responses. More recently, it has been revealed that the perception of the fungal avirulence gene NIP1 elicitor by epidermis cells triggers the induction of genes encoding PR proteins which are expressed in the mesophyll of resistant plants (Steiner-Lange *et al.*, 2003).

Quantitative and qualitative losses due to scald disease have also been studied to evaluate the economical importance of barley-scald interactions (James *et al.*, 1968; Khan and D'Antuono, 1985; Khan and Crosbie, 1988). Losses in yield due to barley scald can be significant and are correlated with the percentage of infection or leaf area diseased by the fungus (Brown *et al.*, 1996; James *et al.*, 1968). Yield losses have been estimated to be as high as 35-40% in severe epidemics. However, losses of 1-10% are probably much more common (Mathre, 1997). In Canada, scald has been particularly troublesome in Alberta and has caused considerable yield and quality losses in susceptible cultivars (Anonymous, 2003). Brown *et al.* (1996) studied the effect of amount of scald disease on yield and quality traits of some lines having different combinations of resistance genes to the scald disease. The amount of disease and area under disease progress curve (AUDPC) were found to be significantly correlated with malt yield, grain size, grain weight, and yield. Lines resistant to scald were reported to have significantly higher grain size and grain weight. Although yield losses are primarily due to reduced kernel weight, the number of kernels per head and the number of heads per plant may also be reduced in severe infections. Kulichova (1997) reported that infections of flag leaves as well as the penultimate leaves had negative influence on both the number and the weight of grains in the ear. Khan and Crosbie (1988) tested the effect of controlling scald with fungicide on malting quality and found that scald damage lowered yield and grain quality, and hence malting quality.

Xue and Burnett (1995) studied the interaction between *R. secalis* and *P. teres* on barley seedlings grown in a greenhouse and in growth chambers. A predominance of net blotch was found over the scald disease even when inoculations with *R. secalis* either preceded or followed the inoculation with *P. teres* by 24 hours. Moreover, an antagonism was observed when inoculum densities were 10^3 - 10^4 spores per ml for each pathogen, wetting periods were 24-48 hours, and incubation temperature was above 12°C. Yitbarek *et al.* (1998) studied variation in Ethiopian barley landrace populations for resistance to barley leaf scald and net blotch. They reported that both diseases were enhanced by the application of fertilizer. Moreover, it was found that populations of barley collected from higher altitudes were more resistant to scald, but more susceptible to net blotch, compared to those from lower altitudes (Yitbarek *et al.*, 1998).

1.5. Scald development and assessment

Development and assessment of diseases are two of the most crucial prerequisites in plant breeding, pathological and genetical studies. Occurrence of a plant disease depends on the presence of susceptible host, virulent pathogen, and favorable environmental conditions (Agrios, 1997). In the presence of the first two factors, optimization of conditions suitable for establishment and development of a disease can lead to an appropriate disease pressure and consequently better differentiation of resistance levels among the host genotypes. Overestimation of resistance may happen when the conditions are not conducive to disease development resulting in improper differentiation of resistance from susceptibility, or escape. On the other hand, extremely favorable environmental conditions coupled with prolonged epidemics creates high levels of disease pressures. Underestimation of resistance due to very high disease pressures may make recognition of intermediate types of resistance difficult as they may be mistakenly categorized into highly compatible reaction classes. Variations among separate studies can often be related to differences among conditions under which the scald disease developed and also to varible evaluation systems that were used by different scald workers (Tekauz, 1995). However, experiments carried out under standardized conditions can facilitate realistic assessment of differential disease responses and comparisons among studies.

1.6. Genetics of resistance against the barley scald disease

Several genes/quantitative trait loci (QTLs) have been identified to condition resistance to *R. secalis*, 17 (14 major genes and 3 QTLs) of which have been named and localized to different barley chromosomes, except Rh5, rh8, Rh10 and rh11 (Abbott *et al.*, 1992; Abbott *et al.*, 1995; Ali, 1975a; b; Backes *et al.*, 1995; Baker and Larter, 1963; Barua *et al.*, 1993; Bjornstad *et al.*, 2002; Bockelman *et al.*, 1977; Bryner, 1957; Dyck and Schaller, 1961a; Evans, 1969; Garvin *et al.*, 1997; Garvin *et al.*, 2000; Graner and Tekauz, 1996; Gronnerod *et al.*, 2002; Habgood and Hayes, 1971; Hansen and Magnus, 1973; Jensen *et al.*, 2002; Riddle and Briggs, 1950; Schweizer *et al.*, 1995a; Spaner *et al.*, 1998; Starling *et al.*, 1971; Wells and Skoropad, 1963; Williams *et al.*, 2001). For the first time, Mackie (1929) reported that a single recessive gene was responsible for the resistance in an unnamed barley variety to *R. secalis* (in Shipton *et al.*, 1974). After 20 years, a barley variety, La Mesita, and its derivatives, cvs. Trebi and Modoc were genetically studied. A single dominant gene was found as their scald resistance factor (Riddle and Briggs, 1950) and later designated as Rh4 (Dyck and Schaller, 1961a). Since then, a number of additional resistance genes have been identified (Table 1-1). Using both morphological markers and translocation interchange stocks, Dyck and Schaller (1961a) localized the Rh3 and Rh4 genes on chromosome 3. Another gene on this chromosome, the Rh gene, together with Rh3 and Rh4 genes were reported to form a complex locus (Dyck and Schaller, 1961b). Starling *et al.* (1970) suggested that Rh, Rh3, and Rh4 could be assumed to be allelic or very closely linked. Bockelman *et al.* (1977) assigned the Rh9 gene to chromosome 4 by trisomic analysis. Two additional scald resistance genes, Rrs12 and Rrs13 from *H. vulgare* ssp. *spontaneum* were found to be located on chromosomes 1 and 6, respectively (Abbott *et al.*, 1992). The dense molecular marker maps of the barley genome (Graner *et al.*, 1991; Heun *et al.*, 1991; Kleinhofs and Han, 2002; Kleinhofs *et al.*, 1993) provided a means for the rapid and accurate localization of resistance genes to scald such as Rhy, Rh2. The Rhy was localized on chromosome 3 (Barua *et al.*, 1993) and Rh2 was mapped on chromosome 1 by restriction fragment length polymorphism (RFLP) markers (Schweizer *et al.*, 1995a). According to a more recent terminology, Rh/rh terminology has been transposed to Rrs/rrs one (Jörgensen, 1992) in which Rrs and rrs represent dominant and recessive resistance genes/loci, respectively. As discussed at the "2nd International Workshop on Barley Leaf Blights", no consensus has yet been reached for adoption of revision of the scald gene nomenclature proposed by Bjornstad *et al.* (2002) yet (Wallwork, 2002). Hence in this review, the gene name appears as it was first named. Abbott *et al.* (1995) reported the map location of the scald resistance gene, Rrs13, from wild barley (*H. vulgare* ssp. *spontaneum*) with respect to RFLP loci on barley chromosome 6H. They also identified two RFLP loci (Cxp3 and ABG485) flanking the Rrs13 locus, which can assist selection for the resistant allele in barley breeding programs. As shown in Table 1-2, various QTLs have been also reported for scald resistance in the barley genome (Backes *et al.*, 1995; Jensen *et al.*, 2002; Spaner *et al.*, 1998). Backes *et al.* (1995) identified a major component of quantitative resistance to *R. secalis* on the long arm of chromosome 2.

Spaner *et al.* (1998) reported three QTLs located on chromosomes 3, 4, and 6 and recently Jensen *et al.* (2002) mapped Qryn3, Qryn4 and Qryn6 on to chromosomes 3, 4 and 6, respectively.

Penner *et al.* (1996) studied the genetic basis of scald resistance in western Canadian barley cultivars. They showed that the majority of scald resistance utilized in western Canada has been based on only two genes/alleles, Rh and Rh2, which could not confer resistance against the scald isolate WRS1860. It was found that the gene in Falcon is the Rh2 derived from Atlas, and the gene(s) in CDC Silky is located within the Rh/Rh3/Rh4 cluster and similar to the Rh gene in Hudson. It was concluded that there is genetic variation for scald resistance in the long arm of chromosome 3 (Penner *et al.*, 1998). Garvin *et al.* (1997) studied a set of advanced backcross barley lines derived from crosses between the cultivar Clipper, and different Iranian and Turkish wild barleys. Single dominant genes, single recessive genes, and a pair of unlinked, dominant genes were revealed to encode the resistance in the studied lines, which consistently exhibited scald resistance. They mapped one such resistance gene on chromosome 5 and since no other scald-resistance genes had been located on this chromosome, they designated it as Rrs14. Cselenyi *et al.* (1998) studied the inheritance of durable resistance of selected spring barley varieties to *R. secalis* and found significant additive effects indicating that the resistance level of barley cultivars may be improved by the hybridization of suitable cultivars. The cultivar Tantangara which is a doubled haploid barley possessing a gene for scald resistance derived from *H. vulgare* subsp. *spontaneum* at locus Rrs13 on chromosome 6 was registered in Australia (Read *et al.*, 1998). Garvin *et al.* (2000) field evaluated a set Rrs14 BC_3 lines and observed significantly less leaf damage on the resistant lines than on their recurrent parent, cv. Clipper. They identified one ideal codominant seed storage protein marker, Hor2, for the Rrs14 locus, which could be practically suitable for indirect selection of the resistance to scald disease. Williams *et al.* (2001) identified a scald resistance gene/allele, Rrs.B87 linked tightly to Rh-Rh3-Rh4 locus complex. They also developed one SSR marker for the scald resistance locus and rationalized its importance for pyramiding resistance genes and durable scald resistance breeding. Reitan *et al.* (2002) used bulked segregant analysis (BSA) to identify molecular markers linked to resistance genes in several breeding lines and their donors. They concluded that introgressed scald resistance genes at the complex Rh locus on chromosome 3 may not be easily pyramided into another genotype possessing resistance gene(s)/allele(s) at the Rh-Rh3-Rh4 locus complex. More recently, inheritance of scald resistance was investigated in two New Zealand barley lines derived from interspecific crosses of *H. vulgare spp. spontaneum* resulting in identification of simply inherited (dominant) gene(s) which could provide barley breeders in western Canada with additional source(s) of resistance to *R. secalis* (Singh *et al.*, 2003a and b).

Basically, resistance of plants to fungi has been reported to be of two kinds: race specific (vertical, specific, complete, seedling, qualitative, major-gene/oligogenic, or transient resistance) and non race-specific (horizontal, general, incomplete/partial, adult plant, quantitative, minor-gene/polygenic, or durable resistance and even, on occasion, tolerance). Various major genes conferring race-specific resistance have been reported (Dyck and Schaller, 1961a; Habgood and Hayes, 1971; Shipton *et al.*, 1974; Starling *et al.*, 1971). In several instances, this type of resistance has been found to be short-lived and was broken down in a few years after cultivar release (Turkington *et al.*, 1998), which has been due to extreme pathogenic variability and the emergence of new pathogenic races (Agrios, 1997; Van der Plank, 1984; Xi *et al.*, 2000a; Xi *et al.*, 2003). In contrast, non race-specific resistance is theoretically considered to operate equally against all pathogenic races and to be more durable (Shaner, 1996; Van der Plank, 1968). This type of resistance usually is governed by a number of minor genes and is considered as a possible solution to the instability of vertical resistance. Development of a more stable broad based resistance has been also achieved through composite crosses and recurrent selection techniques incorporating both sources of resistance e.g. major and minor gene resistance (Saghai Maroof *et al.*, 1983). As some major genes may confer horizontal resistance (Cselenyi *et al.*, 1998) and minor-gene resistance might contribute to vertical resistance components (Parlevliet, 2002; Scott *et al.*, 2000), it is better not to treat major-gene resistance as effectively synonymous with vertical resistance and minor-gene resistance with horizontal resistance. For the sake of simplicity and to avoid any confusions, we suggest to use the genetical terminology of major- and minor-gene resistance.

Slow-scalding, which is the subject of this thesis is a relatively newly recognized phenomenon in the barley-scald pathosystem. It is thought that it can play a crucial role in resistance breeding (Capettini *et al.*, 2002; Turkington *et al.*, 1998). Different sources of slow-scalding resistance have been detected among Canadian and ICARDA/CIMMYT scald resistant barley genotypes through this investigation (Sorkhilalehloo *et al.*, 2002a; Sorkhilalehloo *et al.*, 2000; Sorkhilalehloo *et al.*, 2001; 2002b). Slow-scalding resistance can be classified as a partial resistance because this type of resistance is recognized by a susceptible infection type coupled with a reduction in disease progress or in the percentage of leaf tissue affected by the pathogen (Ginkel and Vivar, 1986; Parlevliet, 1979; Shaner, 1996). Genotypes with partial resistance can exhibit slower levels of disease progress through adequate level of partial resistance components i.e. reduced infection frequency, longer latent period, reduced spore production, or a combination of these (Kari and Griffiths, 1993; 1997). All of these quantitative characters can be used to identify plants with adult-plant resistance (Leonard and Mundt, 1984). Moreover, area under disease progress curve (AUDPC) and apparent infection rate (r) can be appropriate tools in identifying this type of resistance (Brown *et al.*, 1996). Ginkel and Vivar (1986) identified 15 lines with a slow-scalding type of resistance which had been previously considered to be susceptible. These slow-scalding lines had intermediate AUDPC values of 800-2800 and r rates of 0.07-0.13. In contrast, the truly susceptible entries showed AUDPC and r of 2800 and 0.2 and above, respectively. By comparing the components of partial resistance (the parameters of disease progress), different amounts of quantitative resistance to the pathogen can be measured in genotypes having different levels of slow-disease progress (Shaner, 1996). The genetic basis and inheritance of slow-scalding is unknown and needs to be investigated. Xi *et al.* (2000) investigated quantitative resistance to *R. secalis* in some spring barley genotypes. They considered different traits related to resistance/susceptibility of eight Canadian cultivars and stated that the measured variables were significantly correlated to each other. The higher amount of AUDPC, apparent infection rate, infection frequency, lesion length and width, final severity and sporulation, and shorter time to disease onset at 5% severity were found to be significantly correlated to thousand-kernel weight of the plots protected with the fungicide Tilt®. The cultivar Leduc was reported to retard disease development and to be a useful source of durable resistance for western Canada. Although this report resulted in quantification of resistance and its components to *R. secalis* in a small group of western Canadian barley cultivars, more investigations are needed to prove the presence of quantitative resistance among barleys grown in western Canada.

1.7. Scald management

To manage *R. secalis* all possible measures of controlling the disease, i.e. legislative, cultural, biological, chemical, and genetic controls should be considered. The subject of any method of disease control is to stop or reduce the disease build-up and its progress through reducing the amount of initial inoculum and the rate of progress of the epidemic (Parry, 1990).

The spread of seedborne inoculum of *R. secalis* is important in distribution of random infection loci in barley fields and long-distance dissemination of the pathogen (Abbott *et al.*, 2000). Regulatory control can stop the introduction of new (virulent) pathogens from other geographical regions and help barley breeders/pathologists to prevent unwanted resistance failures due to the emergence of new imported races of pathogen. Resistance breeding headquarters like Toluca, Mexico and Lacombe, Canada are unique places where many available resistance genes are exposed to an environment conducive for disease epidemics, and hence provide habitats for introducing and evolving highly virulent/aggressive races of the pathogen. These places are also the centers of breeding program seed distribution to other research sites and eventually to farmers' fields. Cautions should be taken while receiving seed and plant materials from these "hot" places.

Cultural, agronomic, and biological controls have also shown promise in controlling different diseases including the barley scald caused by *R. secalis* (Blakeman *et al.*, 1986; Kulichova, 1997; Mathre, 1997). Since the infested stubble of barley can support sporulation for over 10 months under field conditions, the disease can be controlled by the destruction of this habitat, which is the source of inoculum (Davis and Fitt, 1992). Rotation of barley with other barley cultivars and crops, which are not susceptible to *R. secalis* can be effective in destroying barley residue (Anonymous, 2003). Deep plowing-under of infested crop residues and/or burning them destroys the infested crop remains (Nyvall, 1989). Destruction of infected grass species and volunteer barley reduces the level of inoculum. Kulichova (1997) investigated the efficacy of Trichonitrin (saprophytic fungus *Trichoderma harzianum* Rifai- strain B1) in controlling spring barley scald. The study showed that the bioproduct could be used as an effective treatment method for *R. secalis* primary infection control. Moreover, in addition to acceptable control, the biological treatment enhanced the growth of treated barley plants. Biological control of scald disease should be extended further with the use of more efficient antagonists and hypovirulent strains.

Chemical seed dressings can be an appropriate means of controlling diseases caused by seedborne pathogens (Winter *et al.*, 1998). Seed treatment with fungicides such as Baytan 30 and Vitavax 200 FF can be used to reduce infection from seedborne inoculum of scald (Hollomon, 1984; Kulichova, 1997). Foliar fungicides can provide an appropriate approach to reduce the severity of diseases in the field. Albertini *et al.* (1995) described that flusilazole, propiconazole, and tebuconazole (triazoles), prochloraz (imidazole), and fenpropimorph (a morpholine derivate) are of common ergosterol biosynthesis inhibitors (EBI) group, which can be applied separately or in combination with each other, with different systemic fungicides (e.g., carbendazim), or with contact fungicides like mancozebe and chlorothalonil for scald management. The efficacies of these fungicides were reported to be high enough (70-90%) to ensure the control of *R. secalis*. Foliar fungicides, including benzimidazoles and demethylation inhibitors, have been applied effectively in controlling scald in Europe (Mathre, 1997). In Canada, Tilt® has been registered as a useful systematic fungicide for controlling the foliar diseases of barley (Shaffeek *et al.*, 2000). Gaurilcikiene (1997) reported that Tilt® (propiconazole) applied at the heading stage of winter rye at 0.5 l/ha and Folicur (tebuconazole) at 1.0 l/ha gave the best results in controlling *R. secalis* and promoting a significant yield increase.

Additionally, barley scald has also been reported to be controlled by the use of cultivar mixtures (Kari, 1997; Mathre, 1997; Mundt *et al.*, 1994). Kari (1997) studied reduction in incidence and severity of some foliar barley diseases in duocultures of barley compared to cultivation of pure stands, and showed that the mixtures decreased the severity of foliar diseases such as net blotch and scald. It was concluded that in an area such as Cyprus, where the whole local barley production is used for animal feed and there is no commercial constraint for seed purity, barely mixtures can be an alternative approach for disease control. Abbott *et al.* (2000) found pyramiding of two scald resistance genes into a single line to be more effective rather than using the cultivar mixture (multiline) strategy in which the same two genes were used singly in the mixture of two different lines. This result was in contrast with successful reports on applications of cultivar mixtures in controlling scald disease (Jeger *et al.*, 1981; McDonald *et al.*, 1988). Abbott *et al.* (2000) even reported that mixtures having one or two susceptible components besides the resistant line, suffered no more disease than the resistant line grown alone. The epidemiology of scald pathogen in different environments may address differences obtained between those results. Factors affecting the impact of initial inoculum and subsequent development of the disease may play an important role in causing such differences.

1.8. Molecular studies on barley-scald pathosystem

Barley has been extensively studied as a model crop in genomics (Abbott *et al.*, 1992; Barua *et al.*, 1993; De La Pena *et al.*, 1999; Goodwin, 1988; Graner, 1995; Hayes, 1992; Igartua *et al.*, 2000; Penner *et al.*, 1998; Reitan *et al.*, 2002; Schmidt *et al.*, 2001; Schweizer *et al.*, 1995b; Spaner *et al.*, 1998; Zhang, 1986). Its haploid genome size (5300 Mbp) has facilitated various molecular studies using wide arrays of molecular markers (Ananiev, 1992; Genger *et al.*, 2003; Kleinhofs and Han, 2002; Liu, 1998).

The use of molecular markers can provide useful information for barley breeders and pathologists. In the barley-scald pathosystem, molecular markers have been used to either map genes with major effects (Abbott *et al.*, 1992 & 1995; Barua *et al.*, 1993; Graner, 1996; Graner & Tekauz, 1996; Patil *et al.*, 2003; Sharma *et al.*, 1996) or identify QTLs associated with resistance (Backes *et al.*, 1995; Sayed *et al.*, 2004; Jensen *et al.*, 2002). Furthermore, DNA markers can help breeders to overcome the genotype X environment interaction problems since most of the genes conditioning resistance to *R. secalis* have been reported to interact with the environment (Harrabi, 1996). Finally, such molecular markers will be useful when a breeder is pursuing pyramiding resistance genes in a target genotype (Genger *et al.*, 2003; Graner and Tekauz, 1996). Breeders will be able to transfer resistance genes into new cultivars using marker-assisted selection (MAS) in barley breeding programs (Melchinger, 1990).

Brown *et al.* (1996) developed pairwise combinations of genes for resistance to scald in barley using linked isozyme markers and concluded that pyramiding is a beneficial strategy. Hakim (1996) screened two sets of near-isogenic lines (NILs) by random primers to identify RAPD markers linked to scald resistance genes in barley. He used *H. spontaneum* races *caeserea* and *bargiyyora*, as donor parents (DP) conferring resistance to scald and the *H. vulgare* cultivar Clipper as a recurrent parent (RP). The author concluded that these DNA segments (amplication products) were introgressed from DPs to NILs and may, therefore, be linked to the gene conferring resistance to *R. secalis*. Graner and Tekauz (1996) reported RFLP mapping of an additional dominant resistance gene in the proximal portion of the long arm of chromosome 3 and development of a polymerase chain reaction (PCR)-based codominant marker. It was concluded that the conversion of RFLP marker into a codominant, PCR-based sequence-tagged site (STS) marker could provide a useful means for marker-assisted selection. Garvin *et al.* (2000) used a set of RFLP, isozyme, and seed storage protein markers to map Rrs14. They located Rrs14 scald resistance gene on chromosome 5 between Hor1 and Hor2 loci, approximately 1.8 cM from the latter locus.

Williams *et al.* (2001) described the identification of AFLP and RFLP markers linked to the scald-resistance locus, Rrs.B87, in the barley line B87/14, the mapping of the corresponding resistance gene, and the development of three potentially useful microsatellite markers for use in marker- assisted selection. Reitan *et al.* (2002) found molecular markers to be of higher discriminating value than scald isolates in revealing the nature of introgressed resistance genes. Using both phenotypic data on 189 DH lines and genotypic data from 40 RFLPs, 22 RAPDs, 62 AFLPs, 19 SSRs and five STS markers, three QTLs with no interaction (Qryn3, Qryn4 and Qryn6) were identified and mapped on chromosomes 3, 4, and 6, respectively (Jensen *et al.*, 2002).

1.9.Objectives

This study was undertaken to elucidate the nature of slow-scalding resistance in barley. More specifically, the objectives were as follows:

a) to optimize indoor and field techniques to achieve an effective differential infection of barely genotypes and to develop an effective rating scale for the assessment of scald at both the seedling and adult plant stages (Chapter 2).

b) to study quantitative reactions of western Canadian barley cultivars (WCBC) to mixtures of scald isolates under field conditions and to determine the presence or absence of slow-scalding resistance in WCBC (Chapter 3).

c) to examine qualitative reactions of many barley cultivars with different resistance /susceptibility levels against several Canadian and Mexican isolates of the scald pathogen at seedling stages, and to test for differential virulence and aggressiveness of the isolates studied (Chapter 4).

d) to study the histopathology of healthy versus diseased barley leaf in order to describe the infection process, elemental changes, and disease/resistance related factors in resistant, slow-scalding, and susceptible cultivars, and to investigate the effects of different severities of disease on transportation of vascular exudates through barley leaf auricles (Chapter 5).

e) to investigate the genetic basis and inheritance of slow-scalding resistance in three ICARDA/CIMMYT slow-scalding lines, and CDC Dolly, a Canadian slow-scalding variety (Chapter 6).

1.10. Tables and Figures

Table 1-1. Reported genes conditioning resistance to *Rhynchosporium secalis* in barley.

Variety/line (CI No)	Resistance gene(s)*	Reference
Atlas (4118)	Rh2Rh2	(3,8)
Atlas 46 (7323)	(Rh2Rh2, Rh3Rh3); (Rh3Rh3/RhRh, RhxRhx)	(3,8); (10,1)
Brier (7157)	(RhRh); (RhRh, rh6rh6)	(2,4,8,13); (7)
La Mesita (7565)	(Rh4Rh4/RhxRhx); (Rh^{4}Rh^4, Rh10Rh10/Rh4Rh4, RhxRhx)	(3/1); (7/11)
Modoc (7566)	(Rh4^{2}Rh4^2); (RhxRhx, rhyrhy); (Rh^{2}Rh^2, rh6rh6)	(3); (1); (7)
Osiris (1622)	(Rh4Rh4/Rh3Rh3); (Rh^{4}Rh^4, rh6rh6, Rh10Rh10)	(3,6, 5); (7)
Trebi (936)	(Rh4Rh4); (Rh4^{3}Rh4^3); (RhxRhx, rhyrhy)	(3); (9); (1)
Turk (5611-2)	(Rh3Rh3); (Rh3Rh3, Rh5Rh5/RhxRhx); (RhRh, rh6rh6); (RhxRhx, RhyRhy/rhyrhy)	(4); (3,10); (7); (1)
Wisconsin Win. X Glabron (8162)	(Rh^{3}Rh^3); (RhxRhx, rhyrhy)	(7);(1)
Hudson (8067)	(Rh); (RhRh/Rh3Rh3, RhxRhx)	(8,7,13); (10)
Bey (5581)	Rh3Rh3	(7,5)
Jet (967)	rh6rh6, rh7rh7/Rh^{5}Rh^5	(4/7)
Nigrunundum (11549)	Rrs1, Rrs4	(16)
Nigrunundum (2222)	(rh8rh8); (Rrs2)	(7); (16)
Kitchin (1296), Abyssinian (668)	Rh9Rh9	(4)
CI 4364, CI 4368	rh11rh11	(7)
BC$_3$F$_2$-line 200	Rrs12	(14)
BC$_3$F$_2$-line 30	Rrs13	(14)
BC$_3$F$_2$-line 208	Rrs14	(15)

* Superscripts refer to alleles at the given locus.

CI No: Cereal inventory number, Agriculture Research Service, U.S. Department of Agriculture.

(1) Riddle and Briggs (1950); (2) Bryner (1957); (3) Dyke and Schaller (1961a); (4) Baker and Larter (1963); (5) Wells and Skoropad (1963); (6) Evans (1969); (7) Habgood and Hayes (1971); (8) Starling *et al.* (1971); (9) Hansen and Magnus (1973); (10) Ali (1975a); (11) Ali (1975b); (12) Bockelman *et al.* (1977); (13) Goodwin (1988); (14) Abbott *et al.* (1992); (15) Garvin *et al.* (1997); (16) Patil *et al.* (2003).

Table 1-2. Designated resistance loci for *Rhynchosporium secalis* and their chromosomal locations.

Locus*	Cultivar/line/population in which reported	Reference	Chromosome (ref.)
Rh (Rh1)	Brier, Hudson, Atlas 46	(2), (7)	3HL (18), (14)
Rh^3	Wisconsin Win. X Glabron	(7)	3H (7)
Rrs1	B87/14, Nigrunundum (CI 11549)	(22), (26)	3H (22), (26)
Rh2	Atlas, Atlas 46	(3), (8)	7HS (17)
Rh3	Turk, Atlas 46, Osiris, Bey	(4), (3), (10), (8), (6), (5)	3H (3)
Rh4 ($Rh4^2$/$RH4^3$/Rh^2/Rh^4)	Osiris, La Mesita, Modoc, Trebi	(3), (6), (11); (9), (7)	3H (3)
Rh?	BC-line 53	(13)	3H (13)
Rh5	Turk	(3)	?
rh6 (rhy)	Jet; Osiris, Turk, Modoc, Brier	(4), (7); (1)	4H (12)
rh7 (rh^5)	Jet	(4), (7)	3H (12)
rh8	Nigrunundum (CI 2222)	(7)	?
Rrs2	Nigrunundum (CI 2222)	(26)	7H (26)
Rrs4	Nigrunundum (CI 11549)	(26)	3H (26)
Rh9	Kitchin, Abyssinian	(4)	4H (12)
Rh?	BC-line 11	(13)	4H (13)
Rh?	BC-line 29	(13)	4H (13)
Rh?	BC-line 35	(13)	4H (13)
Rh?	BC-line 59	(13)	4H (13)
Rh10	Osiris, La Mesita	(7)	?
rh11	C.I.4364	(7)	being mapped (25)
Rrs12	BC-line 200	(13)	7H (13)
Rrs13	BC-line 30	(13)	6HS (15)
Rrs14	BC-line 208	(19)	1H (21)
QTL?	DH Igri x Danilo	(16)	2HL (16)
QTL?	DH Igri x Danilo	(16)	3H (16)
QTL?	DH Igri x Danilo	(16)	4H (16)
QTL?	DH Igri x Danilo	(16)	7H (16)
QTL?	DH Harrington x TR 306	(20)	3H (20)
QTL?	DH Harrington x TR 307	(20)	4H (20)
QTL?	DH Harrington x TR 308	(20)	6H (20)
QTL?	DH Ingrid x Abyssinian	(23)	3H (23)
QTL?	DH Ingrid x Abyssinian	(23)	7H (23)
Qryn3	DH Alexis x Regatta	(24)	3H (24)
Qryn4	DH Alexis x Regatta	(24)	4H (24)
Qryn6	DH Alexis x Regatta	(24)	6H (24)

* Superscripts refer to alleles at the given locus. (ref.) = reference.

(1) Riddle and Briggs (1950); (2) Bryner (1957); (3) Dyke and Schaller (1961a); (4) Baker and Larter (1963); (5) Wells and Skoropad (1963); (6) Evans (1969); (7) Habgood and Hayes (1971); (8) Starling *et al.* (1971); (9) Hansen and Magnus (1973); (10) Ali (1975a); (11) Ali (1975b); (12) Bockelman *et al.* (1977); (13) Abbott *et al.* (1992); (14) Barua *et al.* (1993); (15) Abbott *et al.* (1995); (16) Backes *et al.* (1995); (17) Schweizer *et al.* (1995); (18) Graner and Tekauz (1996); (19) Garvin *et al.* (1997); (20) Spaner *et al.* (1998); (21) Garvin *et al.* (2000); (22) William *et al.* (2001); (23) Gronnerod *et al.* (2002) (24) Jensen *et al.* (2002); (25) V. Patil in Bjornstad *et al.* (2002), Patil *et al.* (2003).

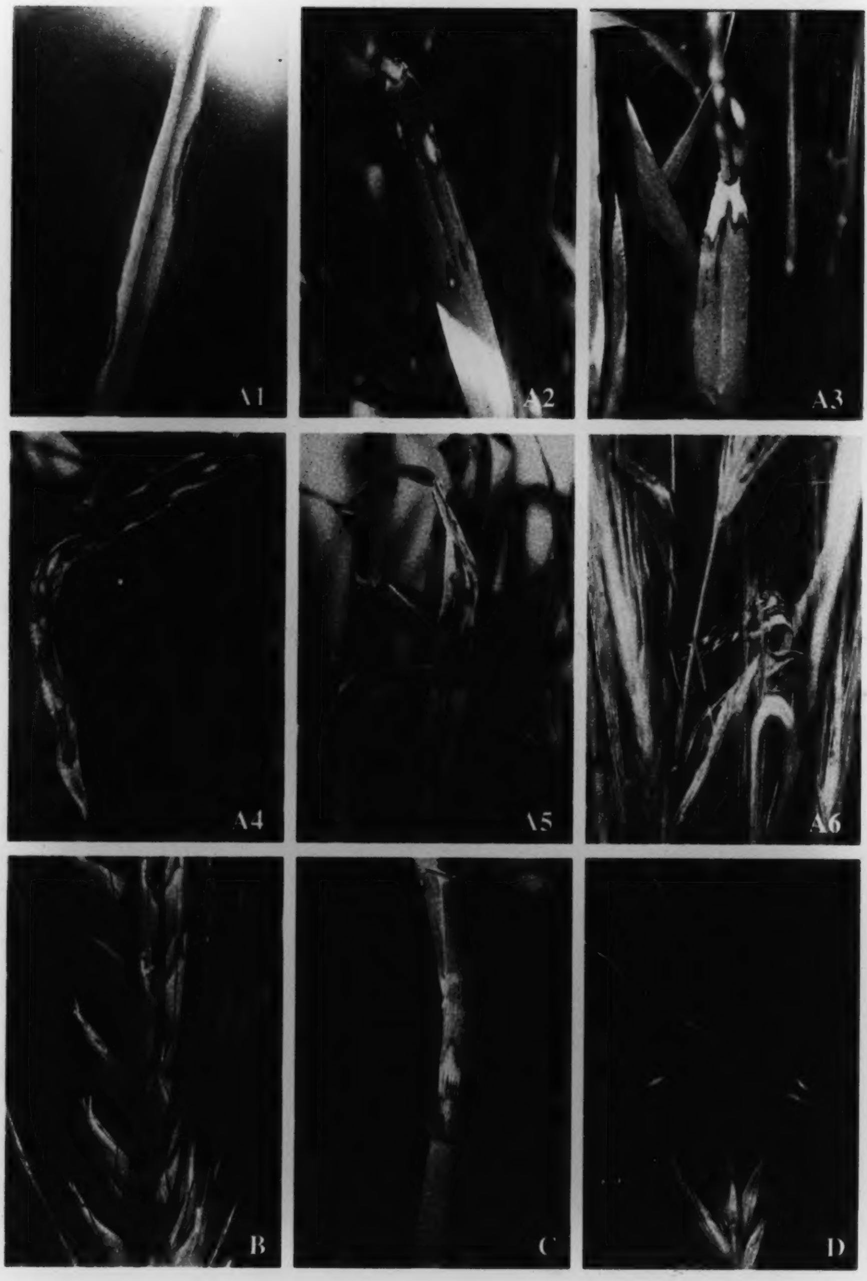
A1
A2
A3
A4
A5
A6
B
C
D

Figure 1-1. Typical symptoms of barley scald disease on leaves (A1-A6), glume (B), leaf sheath (C), and awn (D).

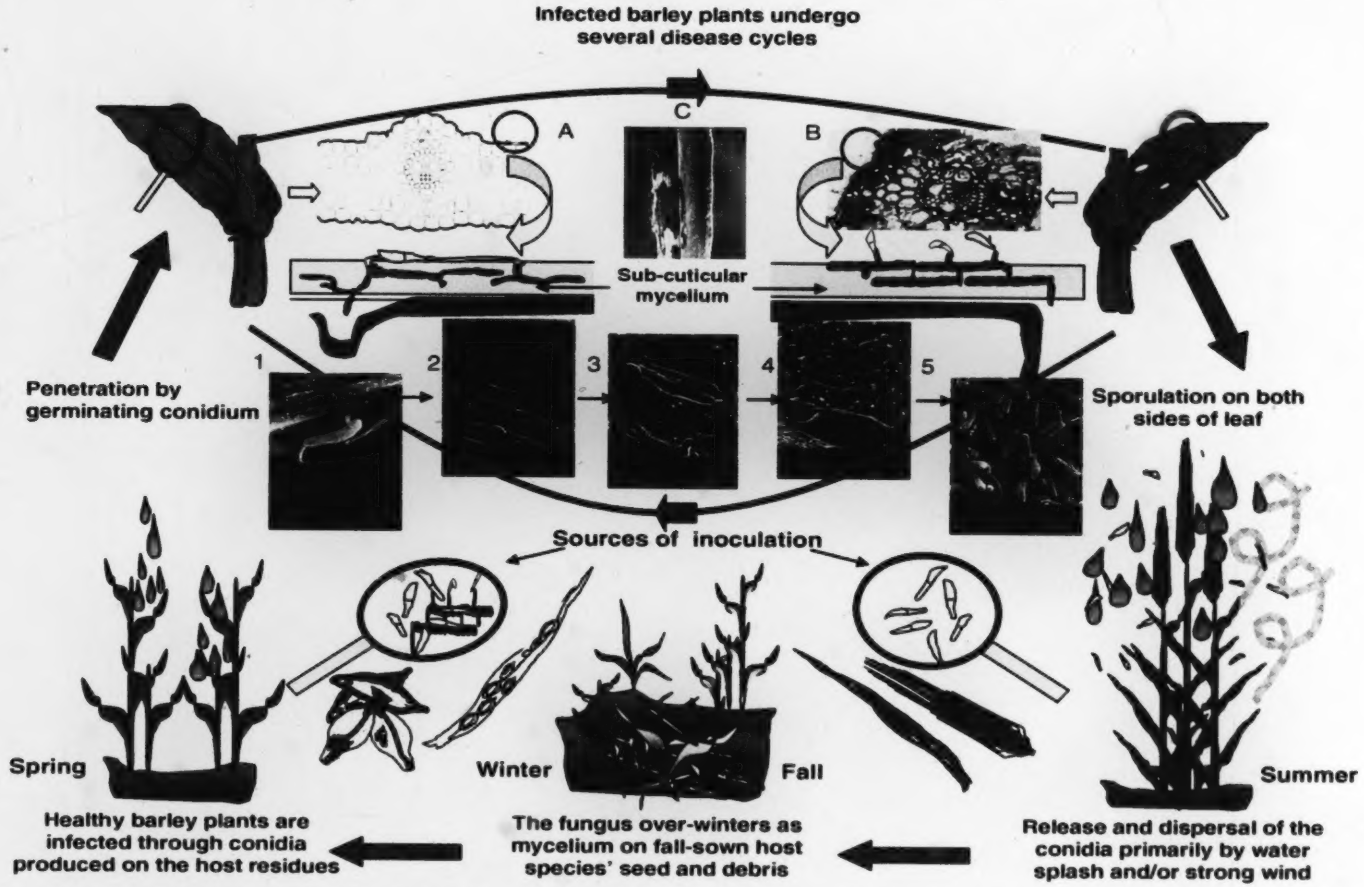
Infected barley plants undergo several disease cycles
C
A
B
Sub-cuticular mycelium
1
2
3
4
5
Penetration by germinating conidium
Sporulation on both sides of leaf
Sources of inoculation
Spring
Winter
Fall
Summer
Healthy barley plants are infected through conidia produced on the host residues
The fungus over-winters as mycelium on fall-sown host species' seed and debris
Release and dispersal of the conidia primarily by water splash and/or strong wind

Figure 1-2. Disease cycle of barley scald caused by *Rhynchosporium secalis* A and B: transverse section of barley leaf blade; C: typical lesion of scald; 1-5 scanning electron micrographs of the infection process.

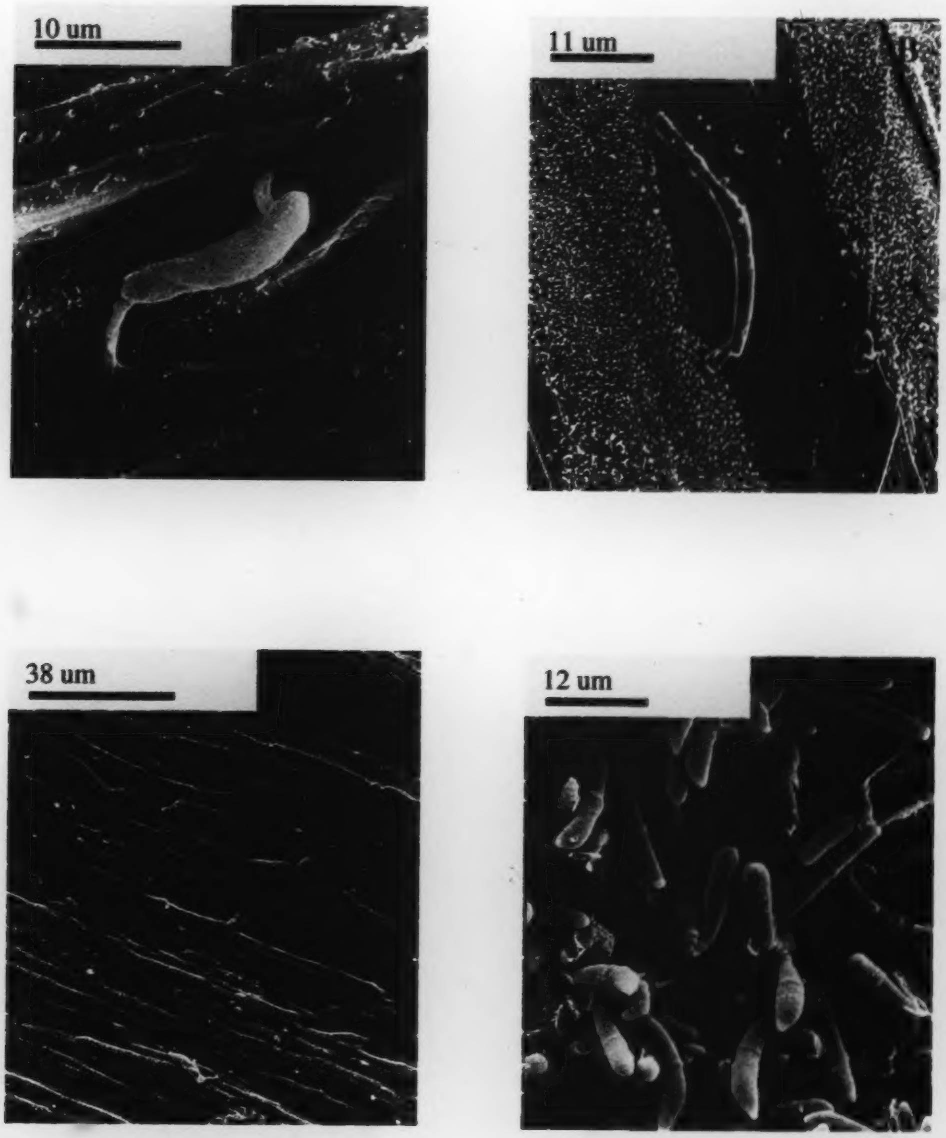
10 um
11 um
38 um
12 um

Figure 1-3. Scanning electron micrographs of *Rhynchosporium secalis* on barley leaf surface. A and B: Germinated conidia C: Sub-cuticular mycelium D: Conidia formation.

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Chapter 2

Techniques for optimal development and assessment of scald under controlled and field environments[§]

2.1. Introduction

Scald, caused by *Rhynchosporium secalis* (Oud.) J. J. Davis, is a major disease of barley in cool and humid areas world wide (Mathre, 1997; Shipton *et al.*, 1974). The effect of environmental parameters on infection of barley by the scald pathogen has been extensively investigated (Ayesu Offei and Clare, 1970; Caldwell, 1937; Ryan and Clare, 1975; Skoropad, 1957). Temperatures lower than 20°C coupled with humid conditions are necessary for initiation of scald epidemics under field conditions (Skoropad, 1957). Similarly, optimum temperature (15-25°C) and duration of leaf surface wetness (>12 h) were reported to be the most important factors in the infection process (the stage from conidial germination to leaf penetration) in controlled environments (Salamati and Magnus, 1997; Skoropad, 1957; Xue and Hall, 1992). To a lesser extent, light could also influence the infection process as it slows the rate of germination of conidia and germ tube growth (Shipton *et al.*, 1974). After leaf penetration, however, the effects of environmental factors on the development of scald symptoms would be minimal with the exception of extremes (temperature >30°C and prolonged wet periods or dry conditions) (Brown, 1991; Skoropad, 1966). The optimal temperature for the establishment of *R. secalis* has been shown to be 18°C (Salamati and Magnus, 1997; Xue and Hall, 1992). Salamati and Magnus (1997) pointed out that their least aggressive isolate was more dependent on the optimum temperature for causing maximum disease level, whilst the most aggressive isolate caused a disease severity of up to 70% of leaf area scalded, irrespective of temperature.

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The environmental conditions at Edmonton (53°/33'N latitude, 113°/28'W longitude and 668 m elevation) and Lacombe (52°/28'N latitude, 113°/44'W longitude and 853 m elevation), Canada, are considered ideal for scald development (Burnett and Helm, 1995). Long term average distribution of precipitation in Alberta shows that 50-60% of annual precipitation occurs during the growing season (http://www.cmc.ec.gc.ca/climate/normals). Thirty-year weather data (1961 to 1991) indicates that the average monthly temperature in the Edmonton area was slightly higher than Lacombe's whereas average monthly relative humidity and wind speed at Edmonton during the months of May, June, July, and August were about 4% and 1 km/h lower than those in Lacombe (http://www.theweathernetwork.com /weather/stats/). Consequently, under field conditions, variations in weather parameters and their influence on scald progress, sometimes result in lower disease pressure at Edmonton than at Lacombe, which may not permit an effective differentiation of susceptible and resistant barley genotypes (Turkington *et al.*, 1998).

Despite the fact that there are numerous studies reported that used a wide range of conditions optimum for the development of scald, there is little discussion about the problems involved in setting-up and carrying out disease assessments under controlled and field conditions. Appendix I provides detailed background information about the conditions and methods which have so far been used for assessment of scald at both seedling and adult-plant stages. In preliminary barley scald inoculation experiments in growth chambers at the University of Alberta, it proved impossible to get typical lesions of scald, or to set-up a routine, uniform seedling test that gave consistent results. Similar difficulties were faced by some colleagues under greenhouse conditions (Dr. T. K. Turkington, Agriculture and Agri-Food Canada, Lacombe, AB, personal communication, 2003).

Artificial inoculation

Field observations have shown that natural inoculation can give rise to an epidemic if there are enough sources of effective primary inoculum uniformly distributed over the studied area coupled with favorable environmental conditions to initiate epidemics (Ayesu Offei and Carter, 1971; Riddle and Briggs, 1950). However, without an effective artificial inoculation, disease severity levels may remain low (Ginkel *et al.*, 2002). A low level of disease may not be sufficient for differentiation of various levels of resistance/susceptibility. Hence, in most studies, artificial inoculation has been preferred over natural epidemic simply due to its advantages in creating adequate and uniform levels of disease (Burnett and Helm, 1995; Turkington *et al.*, 1998). Both spreading infected fresh leaves or infested straw from the previous season and/or spraying of a spore suspension of the pathogen isolate(s) have been applied under field conditions to initiate scald epidemics under favorable conditions (Habgood, 1975; Jenkyn *et al.*, 1989b; Khan *et al.*, 1984; Singh *et al.*, 2003). It has been also considered useful to enhance the amount and rate of infection in experimental plots by application of susceptible spreader rows (Khan *et al.*, 1984) and through increasing the proportion of susceptible genotypes neighboring the plots (Jenkyn *et al.*, 1989a).

Assessment scales

The assessment scales developed and used so far have their own advantages and disadvantages (Couture, 1980; James, 1971; Saari and Prescott, 1975). Assessment scales should provide fast and reliable methods of scoring disease intensity and effectively differentiate amongst various levels of resistance/susceptibility (Campbell and Madden, 1990; Kranz, 1988). The scale also must be readily learned, and used by new raters with a high level of precision, accuracy, consistency, and reproducibility (James, 1971). Lack of any of the aforesaid merits in a rating scale can be considered as being disadvantageous. Subjectivity is also one of the major disadvantages in many scoring systems. Some assessment systems are somewhat subjective as they do not provide enough benchmarks for the intermediate classes of disease intensity, where most readings occur (Davidson and Wolfe, 1985). Distortion is another weakness of several scoring scales where the lowest level of disease intensity is the arithmetic value of 1, and 1 represents the absence of disease (Couture, 1980). Averaging such data and also in situations where the intervals between scores do not correspond to arithmetic distances but rather to logarithmic spans is not logical (Campbell and Madden, 1990). Regardless of the known disadvantages of several scoring systems, there is a tendency to continue using them as they require less time/effort than does adoption of a new system (Dr. T. K. Turkington, Agriculture and Agri-Food Canada, Lacombe, AB, personal communication, 2003).

Necessity for standardization of disease scoring scale

The scoring system can be one of the main obstacles in obtaining consistent and comparable results (Tekauz, 1995). The systems used to assess the reactions of barley genotypes challenged with *R. secalis*, have varied widely. Using different scoring scales, various sets of barley genotypes have been assessed at seedling or adult plant stages and under indoor or field conditions (Ali and Boyd, 1973; Burnett and Helm, 1995; Cromey, 1987; Davidson and Wolfe, 1985; Ginkel and Vivar, 1986; Jörgensen *et al.*, 1996; Jörgensen and Smedegaard-Petersen, 1995; McDonald *et al.*, 1988; Newton *et al.*, 1997; Salamati and Magnus, 1997; Salamati and Tronsmo, 1997; Tekauz, 1991; Xue *et al.*, 1995; Yitbarek *et al.*, 1998). The published methods for measuring the scald disease lack consistency. Regardless of the merits of used assessment scales, there are some disadvantages related to each one of them. Even using the same system, in the lack of standardization, one's perception of a given class could be different from the others (Kranz, 1988; Teng and James, 2002). For example, in an ill-defined descriptive scale "severe infection or small lesion size" might be considered "intermediate or very small", or even "very severe or medium size" by the others. A new assessor may not be able to follow the exact footprint of other scales containing such problematical terminologies. In practice, even in an ill-defined numerical scale, all but the lowest and highest scores could be imprecise (Dr. A. Bjornstad, personal communication). Despite the fact that a particular disease class does not necessarily have consistent severity equivalents over a range of different rating systems, there is no a general agreement between scald workers to apply the same rating scale.

The objectives of research presented in this chapter were to optimize indoor and field techniques for infection of barely genotypes and to develop effective and repeatable rating scales for the assessment of scald at both seedling and adult plant stages.

2.2. Materials and Methods

2.2.1. Indoor and field growth conditions

As shown in Figure 2-1, indoor tests were run under growth chamber and walk-in room conditions at the University of Alberta, where the temperature was controlled by ventilation. The average light intensity was 14.4 klx (measured using a photometer, Model LI-188; Licor Inc., Lincoln, NE 68504) during the day. Polyethylene seeding units (length: 52 cm; width: 26; depth: 6 cm) each with 72 cells were used for planting barley genotypes. A 12-cell space was left empty in the tray for under pan irrigation. Depending on the growth stage of plants, an appropriate amount of water was supplied into the trays three times a week. The water was then soaked up through holes underneath each cell of the pan. For indoor seedling tests, 5 seeds were sown in the seeding unit. Each tray had a transparent plastic cover 20 cm high that sealed over the edges of the tray and enabled maintenance of free water on leaves of the seedlings. Barley seedlings were grown in a growth cabinet at 25±1°C with a photoperiod of 12 hr dark conditions for 2 wk after germination. Metro Mix® (Scotts Horticulture Products Inc. Marysville, Ohio, USA) was used as a soil-less planting medium. A potting mixture was prepared for indoor adult plant tests. The ingredients of the mixture were 1 gallon soil mixture (1 part sand + 1 part peat + 1 part soil), 1 gallon Metro Mix®, 50 g slow-release fertilizer, Nutricote® 14-14-14 (Plantco Inc. Brampton, Ontario, Canada) and 20 g dolomite lime. Similar seeding trays were used for indoor adult plant tests, but the depth of the soil mixture was increased to 12 cm to provide more space for root growth. Two seeds were sown into each cell and after emergence only one plant was kept per cell. Most of the plants were mono-culm and could readily undergo all growth and development stages up to maturity with no morphological signs of nutritional deficiency.

Field evaluations were mainly carried out at Edmonton and Lacombe, Alberta, Canada. Under field conditions, 8-10 seeds were hand-sown in hill plots at approximately 50 cm spacing seeded 0.5 cm deep. Spreader rows of the susceptible cvs. CDC Earl and CDC Kendall were grown all around each of the replicates in experimental tests to enhance the rate and extent of infection.

2.2.2. Inoculation and disease development

Scald culture medium

Rhynchosporium secalis was grown on lima bean agar (LBA), which supports good growth and sporulation of this pathogen (Schein and Kerelo, 1956). Infusion from 62.5 g dry lima beans, 15 g agar, and appropriate amount of sterile distilled water were used for making 1 L of LBA. The infusion was made by gentle boiling of overnight-soaked lima beans for 45 min followed by discarding the beans. The lima bean agar was autoclaved for 25 min and placed at room temperature to cool down to about 50°C before pouring into Petri plates (40 standard 100 x 15 mm).

Single-spore isolation and culturing of *R. secalis*

Isolation and culturing of *R. secalis* is difficult because its slow growth allows almost any contaminant to take over (Schein and Kerelo, 1956). The first step for isolation of *R. secalis* was to collect barley leaves with scald symptoms. As shown in Figure 2-2, margins of young lesions were considered as the best areas of infected leaf tissues for isolation (Gilchrist-Saavedra *et al.*, 1997). The selected areas including edges of the diseased and healthy tissues were cut into small pieces (4 x 4 mm). Thereafter, the work was done under sterile conditions. Using labeled autopsy cassettes, the tissue pieces were wetted in ethyl alcohol for 15- 20 sec followed by surface sterilization in 1% sodium hypochlorite in water for 1 min. Tissues were then rinsed for 2 min in sterile distilled followed by dipping into 70% ethanol for 5 sec. After the second rinse with sterile distilled water, the pieces were taken out with flamed tweezers and blot-dried on a sterile paper towel. Finally, the samples were transferred to lima bean agar plates and sprinkled with streptomycin. The plates were incubated at 16°C in dark. The first sign of growth usually appeared after two weeks of incubation. Therefore, samples with any sign of mycelial growth perior to 2 wk were discarded as possible contaminants (Gilchrist-Saavedra *et al.*, 1997). *R. secalis* growth was picked-off the original plate using a sterile needle and diluted in 1 ml sterile distilled water. The inoculum was uniformly streaked on 1% water agar and incubated at 16°C. One small colony was then taken off the culture medium and deposited on LBA. The culture was incubated at the same temperature for 1-2 wk and harvested for storage in liquid nitrogen. Most cultures of scald were pink in appearance, however black/dark brown colonies were observed in cultures retrieved from liquid nitrogen. At any time thereafter, single-spore cultures were made from cultures derived from liquid nitrogen materials. For production of large amount of inoculum, subculturing of original plates had to be done following harvesting conidia from 2-wk old cultures.

The isolates used in the present study were among more than 250 different single-spore isolates of scald which were collected from different locations in Alberta, Canada, and Toluca, Mexico.

Inoculation and incubation

Different inoculation techniques have been used for studies on the barley-scald pathosystem at seedling and adult plant stages. In this study artificial inoculation was used both under indoor and field conditions. The indoor controlled environment tests included applications of spray inocula of known scald isolate(s). In contrast, a mixture of known and unknown scald isolates was applied to plants in field conditions.

The spray method was used to inoculate plants in the controlled environment facility in both seedling and adult plant tests. After making sufficient numbers of scald culture plates, spores were harvested by gently scratching the surface of plates and subsequent rinsing with sterile distilled water. The resulted conidial/mycelial suspension was then filtered through layers of cheesecloth into a sterile flask. The final volume was adjusted to 1.0 x 10^5 conidia per ml using a haemocytometer and 0.1% Tween 20 was added. Zadoks' growth stages (ZGS) were used to describe the stage during which inocula were applied (Zadoks *et al.*, 1974). Seedlings (4-5) and adult plant in each growth cell were sprayed with approximately 0.5 mL of the inoculum suspension using a 1 L garden sprayer. Practically, spore suspension of the chosen isolate(s) was applied to all leaf surfaces to the stage of run-off. Following inoculation, plants were incubated under humid conditions. For indoor adult plant tests, the day-length was initially set at 16L:8D and subsequent weekly light periods were increased by 12 min per week to a maximum day length of 17L:7D for the first 5 wk, and subsequent weekly dark periods increased by 20 min to a maximum day length of 14L:10D during the last 9 wk before complete maturity. The average light intensity for adult plants was 14.4 klx during the day.

In field studies, freshly harvested infected leaves or infested straw from the previous season was spread on and around the hill plots and/or a spore suspension of the pathogen isolate(s) was sprayed. At Edmonton and Lacombe, infested straw was collected from scald nurseries during the previous year. It was finely chopped/mixed before spreading over barley plants during early stages of stem elongation (ZGS 31-33), approximately 3-4 wk after seeding. Spore-spray inoculation was also applied at ZGS 31-33 in experiments carried out at Edmonton and Lacombe in 2001.

2.2.3. Indoor and field experiments

Starting from 1999, different protocols were examined to initiate seedling infection tests in the indoor controlled environment facilities including greenhouses, growth cabinets, and walk-in rooms at the University of Alberta. The major concerns were to keep the temperature, humidity, and light regimes similar to conditions described in the literature. A trial and error approach was undertaken to elucidate the optimal scald development conditions under a controlled environment. Reaction of a susceptible cultivar, Harrington, to a single isolate of the scald pathogen WRS 1860 was examined under conditions described by Schein (1958), Tekauz (1991), and Xue *et al.* (1995), and then modified based on those outlined by May and Harper (1989). The experiments were repeated twice with and without plastic tents over trays. Three successive tests (Test 1, Test 2, and Test 3) were undertaken to examine the methods of Schein (1958), Tekauz (1991), and Xue and Burnett (1995) under the described conditions. In these tests, Harrington was grown to ZGS 12, 12-13, and 15 and inoculated with 3 X 10^5, 1.0 X 10^6, and 5 X 10^3 spores/mL of WRS 1860, respectively. In Test 1 the post inoculation temperature was set at 17-21°C for 48 h and the seedling were then placed at 20-25°C for 16-19 days in the greenhouse. In Test 2, the inoculated seedlings were incubated at 18°C under dark conditions for 48 h, and were grown subsequently at 18°C for 2-3 wk before rating for disease. In Test 3, conditions were maintained at 23°C during the day and 20°C during the night except for a post-inoculation period when the temperature was kept at 20°C for 48 h following a dry period of 30 min. In Test 1 to Test 3, a high relative humidity (*ca* 95-100 RH%) was created in the test growth cabinet using a regular greenhouse humidifier (mist sprayer) for 48 hr after inoculation. The day length was also adjusted to that required by each published protocols where in the day periods, plants were under a light intensity of about 11.4 klx.

Differential reactions of a set of 10 barley genotypes to six different isolates of *R. secalis* were further tested for both seedling and adult-plant stages under indoor conditions to check the reliability of results. This experiment was conducted three times under the standardized seedling conditions and twice under adult-plant indoor conditions. The results were also compared to those of field tests, where a mixture of the same six isolates was applied to the same barley genotypes as artificial inoculum.

In order to study correlations between indoor seedling and adult-plant tests, corresponding disease reactions of an isolate on a given genotype in both tests were used as pair-wise data. The coefficients of correlation between the data obtained from indoor and field tests were also used to investigate association between the ratings of the corresponding tests. Disease reactions of a given genotype for each isolate in the indoor experiments and the reaction of the same genotype under field conditions were used as pair-wise data. In order to study the overall correlation between disease scores obtained from indoor and field tests, average disease reactions of all the 6 isolates on a given barley genotype and the field reaction of the same genotype were used as pair-data.

Additionally, two case studies were undertaken to elucidate the effects of inoculation treatments on scald severity under controlled and field conditions. Using an indoor experiment at the University of Alberta during 2002, effectiveness of spray inoculum application on development of the scald disease was studied using different barley genotypes and scald isolate treatments at the adult-plant stage (Case Study 1). Effectiveness of infested straw inoculum application on progress of the scald and net blotch diseases was studied in comparison with other inoculation treatments at the adult-plant stage under field conditions (Case Study 2). Both case studies were carried out and analyzed using a split-block model in a randomized complete block design (Steel *et al.*, 1997), and statistical analyses were conducted using SAS procedures (SAS Institute Inc., 1989).

In Case Study 1, the effects of different spray inoculum treatments were investigated on one susceptible (Stander) and two slow-scalding genotypes (UNA 80 and Zavila). The treatments were (1) spray of sterile distilled water, (2) spray of isolate, Lac-

01, (3) spray of another isolate, WRS 1860, and (4) spray of a mixture of Lac-01 and WRS 1860. This experiment was carried out with three replicates. Twenty-five out of approximately 60 plants from each plot (tray) were randomly tested for %LAS (the percentage leaf area scalded) upon application of each spray treatment on each genotype.

In Case Study 2, using one slow-scalding (CDC Dolly) and three susceptible barley genotypes (TR 251, Harrington, and B1602), the effects of infested straw inoculum application on disease progress was compared to that of sprayed spore suspension under Edmonton and Lacombe field conditions during the summer of 2001. Four replicates were used for these experiments. The inoculum treatments at Edmonton were (1) double applications of a spray inoculum of six single-spore isolates of *R. secalis* (09-7, 07-5, B11-4, WRS 1860, Lac-01, and 27-11) at ZGS 31 and 33, (2) spreading a mixture of infested straw from Edmonton and Lacombe scald nurseries from the previous year at ZGS 31 and spraying of the same mixture of spore suspension once at ZGS 33, (3) spreading of the same mixture of infested straw only, (4) uninoculated, and (5) spray inoculation at ZGS 31 prior to treatment with the fungicide propiconazole, Tilt® (200 mL/ac. in 80L water) at ZGS 33. A combination of two different types of overhead irrigation systems i.e. pop-ups and rain-bird sprinklers were applied in Edmonton for two major purposes, which were to reduce the temperature during hot days and to increase the humidity during dry periods. At Lacombe, except for the uninoculated treatment which was replaced with a single spray of inoculum at ZGS 31, inoculation treatments were similar. The plant materials were hand-seeded in hill plots, which were separated by a 0.5 m wide triticale (cv. Pronghorn) buffers to minimize inter-plot interference, except for the fungicide treatment where plants were intended to be exposed to the natural inoculum. Additionally, the effects of infested straw on development of net blotch were studied in comparison with other inoculation treatments that were uninoculated, sprayed with Tilt®, and sprayed with the spore suspension. Scald and net blotch severity (%LAD, the percentage leaf area diseased) and incidence (%I, the percentage of tillers having disease symptom) were then studied.

The slow-scalding cultivar, CDC Dolly, showing an adequate level of field resistance in Canada was developed by Dr. B.G. Rossnagel of the Crop Development Centre, Saskatoon, Saskatchewan (personal communication). UNA 80 and Zavila have been used in ICARDA/CIMMYT barley breeding program as genotypes with slow-scalding resistance, S-SR (Dr. F. Capettini, ICARDA/CIMMYT Barley Breeding Program, personal communication). These slow-scalding genotypes are recognized by their low to intermediate levels of disease progress and rate during sever epidemics (Ginkel and Vivar, 1986).

2.2.4. Disease assessment

To develop effective scald assessment scales applicable to seedling and adult-plant stages, several internationally well-known scientists were surveyed around the globe from Australia, Canada, New Zealand, Norway, Scotland, and Sweden to comment on their scoring systems. Using the information obtained, with respect to the weaknesses and strengths of the studied scoring systems, and based on our experience over assessments of thousands of entries, new illustrated scoring systems were developed for both seedling and adult-plant evaluations. However, these new rating scale was not statistically compared to those used previously by other scald workers as method comparison was not a primary objective.

Disease intensity at the adult-plant stage was measured using a 0-9 scale, where 0 corresponded to no disease and 9 represented a reaction with greater than 50% of lower, middle, and upper leaves diseased. In order to measure disease intensity, both severity and incidence of the disease were visually rated as percentage and integrated into a single digit of 0-9. The percentage leaf area scalded and the percentage of plant tillers infected in each hill plot were visually assessed as disease severity and incidence, respectively, using the scoring guides developed in this study (Tables 2-1 and 2-2, Figure 2-4).

2.3. Results

2.3.1. Disease assessment

The survey on currently applied scoring systems provided a list of the most important advantages, which were to be practical, fast and easy to score, able to differentiate disease classes/response types, and applicable to a large number of individual genotypes (Respondents were Drs. K. Turkington, Agriculture, and Agri-Food Canada; M. Rasmussen, Cereal Breeding Department Landskrona, Sweden; S. Salamati, Kvithamar Research Centre, Norway; A. Bjornstad, Department of Horticulture and Crop Science, Agricultural University of Norway; A. Newton, Scottish Crop Research Institute, UK; H. Wallwork, South Australian Research and Development Institute, Australia; M. Cromey, New Zealand Institute for Crop and Food Research, New Zealand, personal communications). The weaknesses of assessment scales used were discussed earlier in this chapter, and in Appendix I. Novel scoring scales were developed in this study based on two criteria. Firstly, to include advantages of scald scoring systems used by other scald workers, and secondly to avoid the disadvantages attributable to their assessment scales. For the sake of uniformity of scald assessment tests in future, the various infection types (Figures 2-3 and 2-4A) and the percentage area scalded (Figures 2-4B) were illustrated.

Seedling scoring scale

Table A-1 in Appendix I shows different scoring scales, which have been so far used for assessment of scald at the seedling stage. A 0-4 infection response rating scale was developed for evaluation of seedling resistance (Table 2-1). The relationship between reactions of seedlings infected with R. *secalis* isolates and their corresponding lesion types was used to group plant reactions into a few classes: immune (I), resistance (R), moderately resistance (MR), moderately susceptible (MS), susceptible (S), and very susceptible (VS). This scale was then substituted with a numerical system which was easier to use for manipulation of data and statistical procedures.

For the purpose of seedling evaluations, the reaction type of the second leaf was assessed at ZGS 14, 2-3 wk after inoculation according to a scale of 0-4 (Table 2-1). Additionally, HRL and MS represented hypersensitive response-like (score 1) and moderately susceptible (score 2-3) infection types, respectively (Figure 2-3). For the sake of simplicity, this scale was illustrated as a scoring diagram in Figure 2-4A.

In the case of an immune plant response (score 0), no symptom/sign of the scald disease was observed and inoculated plant tissues look quite similar to those of uninoculated ones. On this scale, the resistance class (score 1) was characterized by an incompatible reaction, where the plant tissues challenged with the scald isolate showed a few small necrotic spots associated with the infection by *R. secalis*. Sometimes, these spots looked like HRL symptoms. Compatible reactions contained classes from moderately resistant to very susceptible. The presence of typical lesions of scald was the most significant characteristics of these classes. However, each class was identified with differences in appearance, number, and size of scald lesions on the host tissues (mostly leaves). Indeed, differences between these qualitative classes were considered to be of the quantitative kind where moderately resistant to moderately susceptible seedlings (score 2 or 2-3) showed small lesions (1-5 mm in width) usually confined to the margin of leaves or sheaths with a low level of incidence They occurred anywhere on both surfaces of the leaves. Susceptible hosts (score 3) were recognized by more and larger typical scald symptoms, which sometimes formed coalescing lesions. In the very susceptible reaction (score 4) entire leaves collapsed (Figure 2-5). The severity of lesions in each class varied depending upon the conditions applied to the test, and the type of plant and fungal materials used. Under the optimized conditions, the infection types were characterized as shown in Table 2-1.

Adult-plant scoring scale

Adult-plant assessment was based on the quantification of disease intensity. A modified scale of 0-9, which integrates different aspects of disease intensity (severity and incidence) in one single score, was developed and used for assessment of adult plants/S-SR (Table 2-2).

For measuring disease progress over the course of epidemics at adult plant stages, multiple ratings are required. The first disease rating was done at least 2-3 wk after inoculation and during stem elongation at ZGS 35-39. The last one was done before physiological maturity or at ZGS 85. At both Edmonton and Lacombe, the initial rating was normall made in the middle of July and then every week/two weeks for a total of 2-5 rating dates.

The two upper most (flag leaf, F, and penultimate leaf, F-1), middle leaves (F-2 and F-3), and lower ≥ F-4 leaves were assessed as upper, middle, and lower canopy, respectively. However, data from middle and lower leaves were not kept separate as progress of scald in these canopy levels may not follow the pattern described by previous scoring systems (Burnett and Helm, 1995; Couture, 1980).

The rating was determined on a visual basis for both the disease intensity i.e. disease severity (the percentage of leaf area scalded, % LAS) and incidence (the percentage of tillers having scald symptoms, % TS). Besides the scald lesions, all chlorotic and necrotic areas associated with *R. secalis* were included in the total area scalded. To assign a score to the reaction of a given genotype, the observed %LAS and %I were matched to the range under the defined score. However, when disease severities on defined canopy levels of a plant genotype did not match with corresponding depicted values of the proposed scale, an average of the two closest scores representing the actual %LAS in the canopy levels was used.

The scales were capable of differentiating resistant lines (e.g. Osiris) from moderately resistant (e.g. CDC Dolly or UNA 80 as slow-scalding lines) (Table 2-4), and gave a clear cut off point for eliminating the susceptible and moderately susceptible genotypes. The cut off point was defined as the minimum acceptable level of resistance for determination of acceptance or rejection of breeding lines above which the materials would not be selected (e.g. the score of 5 in our 0-9 scale). For the purpose of screening and describing breeding materials, designation of immune to resistant, moderately resistant to moderately susceptible, and susceptible to very susceptible field reactions to R. *secalis* are shown in Table 2-2. These terms do not necessarily reflect generally determined disease reaction types. Slow-scalding lines were usually classified into the moderately resistant to moderately susceptible grouping in this descriptive system.

The reliability of rating systems developed for both seedling and adult-plant stages was revealed by repeating the experiment with 10 barley genotypes and 6 scald isolates. Results from this repeated experiment could also be used to assess the reliability and repeatability of conditions under which these tests were conducted. Results are described in the section Repeatability/Reliability where effectiveness of the techniques developed for both infection and assessment of scald at seedling and adult-plant stages are presented and discussed.

2.3.2. Optimization of techniques and conditions

2.3.2.1. Controlled environments

Seedling tests

None of the applied protocols (Test 1 to Test 3) alone resulted in either uniform infection or conditions suitable for development of typical lesions of scald. Continuous wetness under optimum temperature and high disease pressure caused partial or total wilting of seedlings, in our preliminary tests. Isolated and/or atypical lesions were observed in some cases. However, trial and error approaches did result in discovery of optimal conditions for development of the scald disease under the controlled conditions used.

Following inoculation, seedlings were kept under a sealed transparent cap at high relative humidity (95-100 RH%) for 24 hr at 15°C in dark and then they were kept for 2 wk at 18/15°C (± 1°C) day/night temperature (in day/night = 12h). At the 3-leaf stage (ZGS 13), the plastic cover was replaced with a hand fabricated tent (length: 52 cm; width: 26 cm; height: 110 cm) using transparent polyethylene sheets and bamboo sticks. Alternate periods of dry (40-60% RH) and wet conditions (60-95% RH) were applied to favor optimum sporulation and development of scald by alternative spraying of Milli-Q H_2O (Millipore water purification system, Billerica, Mass. USA) on the wall and floor of the polyethylene sheet tent twice a day for one week. Conditions were set for a 12 h photoperiod at 11.4 klx light intensity. These methods resulted in formation of typical scald lesions (Figures 2-2, 2-3) and adequate disease pressures, which permitted an effective differentiation of resistance levels among different barley genotypes (Table 2-4).

Adult-plant tests

For indoor adult-plant tests, polyethylene sheet tents similar to those for seedling experiments were made and the same temperature range was used. The average light intensities over the corners and center of the constructed polyethylene tent were approximately 10.2, 14.9, and 18.1 klx at 25, 50, and 75 cm above the tent floor, respectively. The variation of light intensities at different levels of canopy of plants in the constructed polyethylene tent did not influence the intensity of scald as typical lesion and optimal development of scald were observed on all infected barley leaves. To favor disease development and sporulation of the pathogen, Milli-Q® H_2O was sprayed on plants twice a day till the first symptoms of scald appeared approximately 10 days after inoculation. Hereafter, the plants were sprayed once a day till ZGS 83 when terminal severity of the disease was recorded. Persistence of water on walls of the polyethylene tents until the next water spray, was a good indication of continuing high RH.

Case Study 1

The effects of different inoculation treatments were studied on three barley genotypes in the Case Study 1. Analysis of variance for %LAS, shown in Table 2-6, indicates mean squares and their significance for differences among inoculation treatments, genotypes, and Gen x Inoc effects. The results revealed that there were statistically significant differences among genotypes, inoculation treatments, and Gen x Inoc effects at $P<0.01$. Differences among Lac_01 and WRS 1860, and Check were all significant whereas no difference were found between Lac-01 and W x L treatments (Table 2-7). Both WRS 1860 and Lac-01 isolates were virulent on Stander and UNA 80 (Table 2-4) and the latter isolate was found to be more aggressive. The isolate Lac-01 caused significantly more %LAS on Stander as compared to that caused by WRS 1860 (Figure 2-6). The damage caused by WRS 1860 on UNA 80 was not statistically different from check samples whereas severity of disease in the Lac-01 treatment was significantly different from that of check. There were no differences among different levels of inoculation treatments for Zavila.

2.3.2.2. Field conditions

Based on our visual comparisons between irrigated and non-irrigated areas of marginal spreader rows (susceptible cvs. CDC Earl and CDC Kendall) and the data which will be presented in Chapter 3, the application of an irrigation system was considered to be quite effective for both the development of scald and for its continuous progress during the course of epidemics.

Case Study 2

In Case Study 2, the effects of different inoculation treatments were studied on four barley genotypes under Edmonton and Lacombe field conditions. Analyses of variance for the traits studied, %LAS and %I for scald and net blotch (caused by *P. teres* Drechs.), are shown in Table 2-8. The data from Edmonton and Lacombe were not combined since the inoculation treatments at these locations were not the same. Besides, the natural spectra of *R. secalis* and *P. teres* pathotypes were considered to be different at these sites. Inoculation treatments and genotypic effects at both locations were significant. The Gen x Inoc effects were also significant at Edmonton for both diseases and at Lacombe for net blotch severity and incidence. However, the interaction between genotype and inoculation treatments was not significant for scald severity and incidence at Edmonton. Table 2-9 shows means and groupings for the inoculation treatments for %LAD and %I of both diseases. In Edmonton, the application of double spray of scald inocula resulted in significantly better development of scald and lowest levels of net blotch as compared with the other inoculation treatments. In Lacombe, similar results were found with both double spray and spray-straw inocula and to some extent with spray only inoculum. However, with respect to reduction of net blotch intensity, single and double spray inocula were found to be more effective (Table 2-9).

Differences among different levels of the inoculation treatments within each genotype studied are presented in Figure 2-7 and Figure 2-8 for Edmonton and Lacombe. In Edmonton, scald severity and incidence were not different among the inoculation treatments for CDC Dolly indicating its effective field resistance to any inocula applied. A similar situation was observed for TR 251 which was resistant against the net blotch pathogen. In other genotypes/treatments, there were at least one significant difference among the applied inocula and their effect on development of scald and net blotch diseases. Generally, application of double spray of scald inocula was the most effective way to promote scald, whereas application of a mixture of both straw and spray inocula helped establishment and spread of both diseases. The straw only inoculum had only a small effect on increasing %LAD and %I for scald. However, it was observed to be as effective as the spray+straw inoculation treatment on development of net blotch. Both Harrington and TR 251 were found to be less susceptible to scald than B 1602. The application of the fungicide Tilt, effectively controlled scald whereas its effect on net blotch as scald. The effect of natural inoculation for scald was not significant, whereas natural occurrence of net blotch was found to be considerable on the plants. In Lacombe, similarly CDC Dolly appeared to exhibit an effective field resistance resulting in no differences among the inocula applied. TR 251 was also resistant against net blotch showing the lowest amounts of %LAD and %I for net blotch. Double sprays of *R. secalis*, and spray-straw inocula were found to be equally effective in causing high levels of disease on the susceptible cvs. TR 251, Harrington, and B 1602. In contrast to the results obtained in Edmonton, the levels of disease were quite high in all susceptible barley genotypes studied at Lacombe. On the other hand, the level of net blotch for spray-straw and straw only inocula were comparable to those at Edmonton whereas the application of a single spray of Tilt resulted in the lowest levels of net blotch on genotypes studied at Lacombe. At Edmonton net blotch was less sensitive to fungicide treatment than scald. Also, the application of Tilt at Lacombe was not as effective against scald as it was in Edmonton. The levels of scald disease observed on the genotypes treated with Tilt were not significantly different from those recorded from susceptible genotypes inoculated only with application of straw. A single application of spray inoculum resulted in variable levels of scald among genotypes. It was less effective than a double spray in TR 251 and Harrington and as effective as the aforesaid inoculation treatment in B 1602.

The results from correlation analysis revealed that there is no significant relationship between scald and net blotch development at either location studied (Table 2-10). However, the observed negative correlation coefficients between these diseases indicated that as one of these two diseases increases in a given genotype, the severity and/or incidence of the other one decreases.

Repeatability/Reliability

Table 2-3A shows the results of a repeated experiment conducted at both seedling and adult-plant stages under indoor conditions. There were significant differences among both the genotypes and isolates studied. The presence of different barley genotypes and scald isolates with different compatibilities is considered a crucial prerequisite for examining differentiation among plant genotypes/disease pathotypes or repeatability of any plant disease test. The effect of genotype x isolate was consistently significant at $P<0.01$ in all tests (Table 2-3). Table 2-4 presents differential reactions of 10 barley genotypes to 6 isolates of the scald pathogen at seedling and adult-plant stages. Average response to isolate 09-7 Duke did not differ among the barley genotypes tested. Also among the barley genotypes, Osiris and Zavila were resistant to all isolates studied. However, using the developed assessment systems, the reactions of barley genotypes were observed to be highly repeatable for barley seedlings and adult-plants studied under standardized indoor conditions. As shown by Table 2-3B, the test of homogeneity of variances showed that the seedling tests were run under quite uniform conditions as revealed by non-significant variances of Gen, Iso, Gen x Iso, and error terms. Similar results were obtained while testing the uniformity of variances of the indoor adult-plant tests.

Similarly, correlation analyses revealed that the ranking of genotype by isolate means in all indoor tests were highly correlated within (intra-relationship: $r \geq 0.96$, $P < 0.001$) and between (inter-relationship: $0.79 \geq r \geq 0.83$, $P < 0.001$) seedling and adult-plant tests (Table 2-5A). Intra-relationships were another indication of repeatability of the tests, whereas inter-relationships showed association between the seedling and adult-plant reactions. Table 2-5B shows the correlations between indoor seedling and adult-plant (Ad-Sd), field adult and indoor adult (AdF-AdI), and field adult and indoor seedling (AdF-Sd) tests studied based on the average disease reactions of 10 barleys challenged with 6 scald isolates. The results showed no relationship between indoor adult and seedling tests, and also indoor and field results for the isolate 09-7. The lack of any relationships between different tests for this isolate was simply because this isolate did not differentiate among the genotypes studied. Other coefficients were significant and showed that there were significant relationships among plant growth stages, and indoor and field tests.

2.4. Discussion

Conditions conducive to development of scald

One of the main objectives pursued in this study was to address problems involved in creating a proper scald infection under indoor and field conditions. This has not been addressed well in the literature. In order to address problems involved in setting a uniform infection, a protocol was developed using which the seedling and adult plant tests were carried out successfully. In these studies factors like appropriate temperature, humidity and its duration, artificial inoculation with virulent/aggressive scald isolates, stages of inoculation, post-inoculation conditions, dates of rating, application of fast-scalding spreaders, and scales of rating were considered important for an effective disease development and assessment.

Seedling tests

Lack of optimal conditions for uniform inoculation and effective development of scald at the seedling stage results in failure to obtain differential infection types (Drs. A. Bjornstad and M. Cromey, personal communications). In addition to the aforesaid problem, and in the absence of a consensus on using a standardized evaluation system, the seedling test has become a tricky area to work with in studies of the barley-scald pathosystem. Optimal condition for a routine seedling test were identified at the University of Alberta indoor facility which resulted in a uniform development of scald. This was evidenced by obtaining repeatable uniform infection on a set of barley genotypes infected by several scald isolates investigated in this study. Discussion about the repeatability of data is given later. Important factors contributing to the success were maintenance of optimal temperature and humidity during the whole infection process until development of typical lesions of scald. Most plant growth indoor facilities are equipped with ventilation systems to reduce the temperature, and to exchange inside and outside air through air currents (Anonymous, 2003). It was concluded that, although air currents could favor spore dispersal as evidenced by Stedman (1980), they would also cause evaporation of water from plant surfaces which could reduce infection. The application of polyethylene coverings was considered useful in preventing evaporation of plant surface water due to ventilation, maintaining humidity longer, and reducing the light intensity, which all favored *R. secalis* development.

Indoor adult-plant tests

Assessment of both seedling and adult-plant reactions to scald in the walk-in growth chambers proved to be fast, efficient, and reliable. Besides a routine seedling test, this system would enable running at least three sets of adult-plant evaluations for numerous barley breeding lines during a year, even when field evaluations are not feasible. Additionally, feasibility of indoor applications of different spectra of the virulent isolates of *R. secalis* rather than only those of local origin, would help us to breed for durable resistance, where the import of isolates from other geographical districts is prohibited and/or national/international evaluations problematical.

Case Study 1

It has been shown that different isolates virulent against a given genotype may show different aggressiveness capabilities, causing similar disease reaction types at the seedling stage, but different quantitative losses on the adult-plant stage of the same host. (Van der Plank, 1984). Hence, choice of isolates should be considered as an important component of proper inoculation and development of scald. Basically, Case Study 1 demonstrated both differences among different scald isolate treatments, and differential reactions of a set of barley genotypes to the isolate treatments applied.

It was shown that the two scald isolates used in Case Study 1, exhibited different pathogenicity patterns and differentiated among the barley genotypes studied. Such results showing differences in virulence and aggressiveness of different isolates of a given pathogen should assist plant pathologists in making effective inocula for screening genotypes depending on the plant's growth stages. The components of each inoculum must have enough virulence/aggressiveness to differentiate among genotypes with different levels of resistance/susceptibility. The hosts also exhibited different responses to the isolates tested. Lines with S-SR sustained less damage to their photosynthetic area evidenced by significantly lower %LAS as compared to that of the susceptible check, cv. Stander. It was concluded that slow-scalding genotypes may suffer less yield loss. However, field experiments with large plots in different locations/years are required to study yield losses in susceptible genotypes compared to lines with S-SR.

Repeatability/Reliability

To avoid problems with respect to repeatability, it is crucial to conduct seedling tests under highly uniform and reproducible conditions (Dr. H. Wallwork, personal communication). The results from repeated tests conducted in this study, confirmed that our indoor conditions were highly reliable as evidenced by the results shown in Table 2-3 and 2-5. Within each indoor test for seedlings or adult-plants, all the corresponding sources of variation were homogenous. Additionally, highly significant coefficients of correlation were found among the tests, all indicating that the test conditions were highly uniform and reproducible for optimal evaluation of scald at both seedling and adult-plant stages.

Field tests

Of the three components of plant disease triangle, environment is the only one that is usually not modifiable under field conditions (Aust and Kranz, 1988). However, the application of irrigation system was considered useful for alleviating potential problems caused by high temperatures and dry periods at Edmonton. Since hot and dry periods could alter growth of *R. secalis* and reduce scald development, and differences among locations/years with variable number of hot/dry days may cause variation among different experiments, application of such a system would help researchers in creating uniform and progressing disease epidemics with comparable data.

Case Study 2

In Case Study 2, the main objectives were to investigate the effectiveness of artificial inoculation and to study the effects of straw inoculum on creating scald and net blotch diseases under field conditions at Edmonton and Lacombe. Artificial inoculation was found to have significant effects on scald disease progress at both locations. However, it was shown that spray of inoculum had several advantages over the application of infested straw including lowering the development of net blotch and its impact on scald. The straw inoculum was shown to significantly increase the intensity of net blotch. Hence, when using infested straw inocula in a scald nursery, care must be taken to avoid problems associated with the presence of other pathogens of barley like net blotch and spot blotch. Another disadvantage of straw inoculum is that it may not have the same effectiveness as that of original inoculum used to infect it. Even if inoculated with a mixture of different isolates of the pathogen, an infected host may neither necessarily reflect the pathogenicity of the isolates applied to infect it, nor carry the same number of isolates used in preliminary inoculum to the next season. Therefore, when the aim of investigation is to study only one disease, it is advantageous to prevent the occurrence of other diseases by appropriate control measures, or to choose plant materials resistant to unwanted diseases.

It is also problematic if resistance to scald is not independent from those of other diseases which may naturally occur in the disease nursery, a case in many Canadian barely cultivars, which are susceptible to both net blotch and scald (Anonymous, 1989-2004). No association between *R. secalis* and *P. teres* was found in this study as revealed by the correlation study. The effects of net blotch disease on scald disease development seemed to be a matter of competition for the remaining photosynthetic area rather than to be of an antagonistic nature. This was in agreement with the findings of Xue and Burnett (1995) who found no antagonism between scald and net blotch under field conditions. Similar results were also derived from a study on different generations of Composite cross II indicating that multiple resistance for both diseases is unlikely to be hindered by negative associations between resistance to the diseases (Saghai Maroof *et al.*, 1983). In a similar study, Jenkyn *et al.* (1989b) found no interaction between scald and powdery mildew and reported that severe scald could lessen the amount of susceptible tissue available for infection by powdery mildew.

Disease assessment

As explained earlier in this chapter and also in Appendix I, the scoring systems which have been so far used have some disadvantages. Also, there has been no universal agreement among workers to use the same scoring system(s) for evaluation of scald disease. It is hoped that the scoring system developed here will prove useful for other workers and make studies on the barley-scald pathosystem more comparable.

Standardization of scald assessment scales for both seedling and adult plant stages could play an important role in generating precise, accurate, comparable, and reproducible data. To achieve such a goal and in order to assess thousands of barley genotypes both at the seedling and adult plant stages consistently, standardized scoring diagrams were proposed in the scoring systems developed. The illustrated scald rating systems provided in this study could effectively facilitate diagnosis of the type/morphology of lesions and qualitative ratings of infected barley plants. This could help raters to assess the disease in a fast and comparable fashion. Procedures for the scald assessment system proposed for both seedling and adult-plant evaluations were developed in order to meet some degree of standardization in the disease evaluation methods for future works on scald. Based on related literature published, so far, there has been no attempt to develop/publish such scald scoring guides for both seedling and adult plant assessments.

The developed rating scales were used for rating several thousand seedling and adult-plants studied for the purpose of the present thesis research. The proposed assessment scales proved to be practical, simple, differential, and reliable in providing reproducible scores. As discussed earlier, repeatability of the seedling and adult plant conditions was determined using the scoring systems developed in this study. Hence, it can be concluded that the scores obtained within each test were reproducible as well.

In this study, the classes/scores within each of the two scales were developed to be closer to the actual response of a plant to disease. They were also attempted to be efficient in screening for different resistance levels. However, the developed systems were not statistically compared to the other available scales used for assessment of scald. Scores in the seedling scale represented infection types and could classify different levels of compatibility to *R. secalis*. Also, scores in the adult plant scale represented the quantitative reactions of the barley genotypes studied and were considered useful to screen for scald resistance with a higher probability of identifying lines with S-SR. Comparing the scales of 0-9 used before to that developed in this study, a plant with a LAS $\leq$ 25% and only isolated scald lesions on the upper canopy (LAS $\leq$ 1%) would receive a disease score of 5 in the proposed scale. However, it would receive a score of 4 in the scale proposed by Couture (1980) and modifieds by Burnett and Helm (1995), whereas in Couture's scale, a plant with a LAS of 50% in the lower canopy could also receive a score of 4. Hence, the proposed scale is considered to provide, more benchmarks for intermediate levels of resistance. In breeding for resistance, the major concern should be to differentiate between resistance levels, an area closer to the score of 0. The proposed scoring scale was intended to facilitate identification of intermediate types of resistance among barley lines and to lower the chance of losing slow-scalding lines by the application of previous rating systems. For the purpose of screening a barley germplasm for resistance to *R. secalis*, this scale could result in a fast elimination of undesirable genotypes and appropriate selection for S-SR.

The levels of susceptibly defined for different levels of canopy in the other two scales are found to be unreal in many cases as compared to those which are usually seen in the field conditions. In fact, based on personal observations (unpublished), there are many exceptions to match a score with severities defined at each level of canopy. For example, slow-scalding genotypes usually do not show disease development as a disease pyramid, where the oldest leaves are the first and most severely infected. In this case, isolated to light disease intensities may occur at mid point of the plant or the two uppermost leaves with no defined level of disease (25-50%) on the lower leaves. That is why the middle and lower canopy levels in the developed scale were jointly considered to reduce the number of cases with exceptional distributions of disease symptoms.

HLR spots observed in this study were associated with resistance and could be considered as a symptomatic infection type in seedling tests. The HRL symptoms were in the form of small, brown, and usually round to oval necrotic spots. The HRL spots could not be classified as being physiological since the uninoculated plants did not show these symptoms under the same conditions.

For breeding purposes two effective ratings are suggested. The first effective rating should differentiate between susceptible/fast-scalder and resistant genotypes at the middle of epidemics when differences between different levels of resistance are masked. And, the second one must be done when slow-scalding lines can be separated from completely resistant genotypes towards the end of epidemics.

Although, a high level of association was found between %LAD and %I, there were plants with intermediate levels of resistance with similar %LAD and different %I.

Hence, it is suggested that disease incidence should also be considered important in adult plant assessment of scald.

Relationship between seedling and adult-plant tests

In the present study and with respect to the objectives of this thesis research, both seedling and adult-plant reactions of barley genotypes to *R. secalis* were studied. According to the results obtained, there were strong relationships between reactions of these two stages of the barley plant studied to scald as evidenced by significant coefficient of correlation (0.79- 0.83, $P < 0.001$). However, only about 64% of total variation between the two tests could be explained by these coefficients. This source of discrepancy is likely because of slow-scalding genotypes which showed different reactions at their seedling and adult-plant stages. According to our findings (Table 2-4), a slow-scalding line may be resistant at both plant growth stages (CDC Dolly x 07-5, UNA 80 x B11-4) or be susceptible at the seedling and resistant at the adult stage (CDC Dolly x Lac 01, UNA 80 x Lac 01). It also may be susceptible at the seedling stage and show an intermediate type of reaction at the adult plant stage (CDC Dolly x B11-4). There were also cases in which no relationship could be found between the seedling and adult plant tests. For example, the coefficients of correlation between seedling and adult-plant tests for the scald isolate 09-7 were not statistically significant. The differences among the virulence/aggressiveness of pathogen isolates could be a cause for such differences. In reference to the literature, it has also been shown that resistance or susceptibility at the seedling stage may not necessarily correspond to resistance or susceptibility at the adult plant stage (Abbott *et al.*, 1991; Davidson and Wolfe, 1985; Xue *et al.*, 1995).

Complementary information can be derived from results obtained from both seedling and adult plant tests useful for a proper management of the disease. However, most studies on the barley scald disease have focused on either seedling or adult plant tests. While seedling tests could result in a fast and reliable data on major-gene resistance, there is a tendency among scald resistance breeders to rely only on field evaluation tests rather than to spend time and energy for running seedling tests since stability over time and the maintenance of isolates are considered major limitations of the latter (Dr. M. Rasmussen, personal communication).

2.5. Tables and Figures

Table 2-1. Proposed scale of 0-4 for measuring the infection type in barley at the seedling stage.

	Score				
Scale (#)	**0**	**1**	**2**	**3**	**4**
Reaction type *	I	R	MR-MS	S	VS
Characteresitics	NS	Very small	Few small lesions	Several discrete large lesions	Large coalescing lesions
size of symptom	-	≤ 1mm	1-5 mm in width	> 5 mm in width	> 10 mm in length
Color / Shape	-	Brown spots, round to oval	Greyish blue/green with/without brownish margin, oval	Grey with brownish margin, oval	Greyish blue with chlorotic /necrotic areas,irregular
Location on the leaf	-	Anywhere	Often on the leaf margins	Often in the centre of leaves	Anywhere
Severity	-	LAS = 0	LAS ≤ 5	LAS > 5	LAS > 30

* I: immune, R: resistant, MR-MS: moderately resistant to moderately susceptible, S: susceptible and VS: very susceptible.
Abbreviations: NS = no symptoms; LAS: % leaf area scalded.

Table 2-2. Proposed scale of 0-9 for measuring slow-scalding in barley at the adult plant stage.

	Score*									
Canopy levels**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Upper	0	0	0	0	0	0-1	2-5	6-15	16-35	≥ 36-50
Lower and Middle	0	0-1	2-5	6-10	11-15	16-25	26-35	36-50	>50	>50
Maximum %I	0	1	5	10	20	30	40	50	75	100
Descriptive score***	<-------- I-R -------->			<------ MR-MS ------>			<------------- S-VS ------------->			

* The rating is based on visual assessment of disease intensity i.e. disease severity (the percentage of leaf area scalded, % LAS) and incidence (maximum percentage of tillers having scald symptoms, % I).

** Upper represents flag and penultimate leaves.

*** Proposed resistance classification for selection purposes. I, R, MR, MS, S, and VS represent immune, resistant, moderately resistant, moderately susceptible, susceptible, and very susceptible field reactions to barley scald, respectively. Slow-scalding lines are classified into MR-MS group.

Note: When disease severities on defined canopy levels of a plant genotype do not match with corresponding depicted values in this table, an average over two closest scores representing actual %LAS in the canopy levels can be used.

Table 2-3. (A) Mean squares for the disease score of 10 barley genotypes challenged with 6 separate single-spore isolates of *Rhynchosporium secalis* at 3 seedling (Sdlg 1-Sdlg 3) and 2 adult-plant (Adlt 1- Adlt 2) tests, (B) Chi-square values (χ^2) for the test of homogeneity between different indoor tests.

A

		Experiment				
S.o.V‡	**df**	**Sdlg1**	**Sdlg2**	**Sdlg3**	**Adlt2**	**Adlt3**
Rep	2	0.022	0.001	0.104	0.068	0.156
Gen	9	18.269**	17.455**	17.807**	75.508**	79.71**
Iso	5	26.921**	26.345**	25.819**	57.488**	59.689**
Gen x Iso	45	1.918**	1.769**	1.688**	7.246**	7.525**
Error	118	0.058	0.031	0.073	0.164	0.25

B

	Chi-square	
S.o.V	**Seedling**	**Adult-plant**
Gen	< 1.0	< 1.0
Iso	< 1.0	< 1.0
Gen x Iso	< 1.0	< 1.0
Error	3.9	3.3

** Significant at P<0.01.

‡ S.o.V: Source of variation, Rep: Replicate, Gen: Genotype, and Iso: Isolate.

Note that error mean squares were not significantly different according to χ^2 test of homogeneity of error variance (Steel *et al.*, 1997).

Table 2-4. Average differential reactions of different barley genotypes at seedling and adult plant stages under controlled and field environments.

	Scald single-spore isolate *						
Barley Genotype	**09-7 Duke**	**07-5 Harr**	**B11-4 Dolly**	**WRS 1860**	**Lac-01 Earl**	**27-11 Earl**	**Score ** at Field Ave±Std**
Brier	0 (0)	0 (1)	5 (2.5)	3 (3)	6 (3)	1 (2)	5.3±2.1
Osiris	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	2 (1)	1.0±1.3
CDC Dolly 6	1 (1)	1 (1)	5 (2.5)	2 (1)	4 (3)	2 (1)	2.5±2.2
RFLP Harrington	1 (0)	5 (3)	7 (4)	6 (3)	6 (4)	7 (4)	5.8±2.4
Stander	1 (0)	5 (3)	6 (4)	5 (3)	8 (4)	7 (3)	7.3±1.4
TR 251	1 (0)	4 (3)	5 (3)	5 (4)	6 (4)	7 (3)	6.5±1.1
UNA 80	2 (1)	4 (2.5)	1 (2)	2 (3)	4 (3)	4 (2.5)	4.1±1.1
Zavila	0 (0)	2 (1)	0 (0)	0 (0)	2 (2.5)	1 (1)	1.1±0.9
CI1240/Foma//CI16239.15D	0 (1)	3 (2.5)	1 (2)	1 (3)	5 (4)	2 (4)	2.8±1.5
97D/H-198	0 (0)	8 (4)	8 (4)	7 (3)	9 (4)	8 (4)	7.4±1.2

* Reaction of a set of 10 barley genotypes to 6 different single-spore isolates of scald under controlled environment where adult plant scores, 0-9 scale precede those from the seedling test (0-4 scale).

** Data from Chapter 3 and 6. Average (Ave.) and standard deviation (Std) of field responses of the genotypes studied at adult plant stage using the same 0-9 scale in Edmonton during 2001-2002.

Table 2-5. Pearson's coefficients of correlation between (A) different seedling (Sdlg 1-Sdlg 3) and 2 adult-plant (Adlt 1- Adlt 2) tests, and also (B) between average disease reactions of 10 barley genotypes challenged with 6 scald isolates under indoor and field conditions.

A

Experiment	Sdlg1	Sdlg2	Sdlg3	Adlt1
Sdlg2	0.98			
Sdlg3	0.97	0.97		
Adlt1	0.82	0.82	0.83	
Adlt2	0.79	0.80	0.80	0.96

All coefficients were significant at P<0.001.

B

	Pair-wise comparisons ‡		
Isolate	**Ad-Sd**	**AdF-Sd**	**AdF-AdI**
Iso 1: 09-7 Duke	0.38	-0.33	0.19
Iso 2: 07-5 Harr	0.94 **	0.81 **	0.73 *
Iso 3: WRS 1860	0.73 *	0.85 **	0.94 **
Iso 4: B11-4 Dolly	0.91 **	0.91 **	0.84 **
Iso 5: 27-11 Earl	0.70 *	0.72 *	0.82 **
Iso 6: Lac-01 Earl	0.85 **	0.70 *	0.93 **
Average (Overall)	0.86 **	0.86 **	0.94 **

‡ Ad-Sd: Adult by seedling for indoor tests, AdF-Sd: Adult filed by indoor seedling tests, and AdF-AdI: Adult indoor by adult field tests.

* and ** Significant at P<0.05 and P<0.01, respectively.

Table 2-6. Analyses of variance of the percentage leaf area scalded (%LAS) assessed in Case Study 1, where the effects of different inoculation treatments were studied on three barley genotypes.

S.o.V‡	df	Mean square %LAS
Rep	2	8.2
Inoc	3	884.5**
error 1 (Inoc*Rep)	6	10.6
Gen	2	2939.2**
error 2 (Gen*Rep)	4	5.8
Gen*Inoc	6	416.3**
error 3	12	15.2

‡ Rep, Inoc, and Gen represent replicate, inoculation treatment and genotype, respectively.
** Significant at P < 0.01.

Table 2-7. Means and grouping for the inoculation treatments studied in Case Study 1.

Treatment\Trait‡	%LAS	
Check	0.0	C
WRS 1860 (W)	9.0	B
Lac 01 (L)	21.4	A
WxL	19.3	A

* %LAS: The percentage leaf area scalded.
‡ Check, W, L, and W x L represent uninoculated and inoculated with isolate WRS 1860, Lac-01, and a mixture of both isolates, respectively.
Means with same letter are not significantly different according to Tukey's HSD at P< 0.05.

Table 2-8. Analyses of variance for %LAD and %I which represent the percentages leaf area scalded and disease incidence, respectively for scald and net blotch at Edmonton and Lacombe field conditions, where the effects of different inoculation treatments were studied on four barley genotypes (the Case Study 2).

		Edmonton				Lacombe			
		Scald		Net blotch		Scald		Net blotch	
S.o.V‡	df	%LAD	%I	%LAD	%I	%LAD	%I	%LAD	%I
Rep	3	24.0	96.9	121.4*	421.4**	256.7*	143.8	14.2	38.9
Inoc	4	783.5**	7375.6**	646.2**	8433.7**	823.3**	1760.5**	114.1**	312.7**
error 1 (Inoc*Rep)	12	15.3	124.9	33.0	320.2	116.4	122.4	8.9	24.7
Gen	3	962.9**	4829.7**	901.7**	7153.9**	2215.6**	4921.0**	229.3**	568.0**
error 2 (Gen*Rep)	9	10.7	58.5	46.2	100.8	108.1	22.6	5.0	26.3
Gen*Inoc	12	197.6**	1292.4**	68.3*	1095.8**	111.7	150.3	48.9**	133.5**
error 3	36	20.2	56.0	33.0	72.3	83.6	143.8	13.4	27.0

‡ Rep, Inoc, and Gen represent replicate, inoculation treatment and genotype, respectively.
* and ** significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 2-9. Means and groupings for the inoculation treatments studied in Case Study 2, where %LAD and %I represent the percentages leaf area scalded and disease incidence, respectively, for scald and net blotch.

		Disease‡			
		Scald		Net blotch	
Location	Treatment*	%LAD	%I	%LAD	%I
Edmonton	Sp_Sp	17.4 A	47.5 A	0.7 C	0.9 D
	Sp_St	10.9 B	35.7 B	16.7 A	55.4 A
	St	4.2 C	9.4 C	15.2 A	44.3 B
	NI	2.0 C	1.2 D	9.1 B	11.6 C
	Fg	0.8 C	0.4 D	8.4 B	17.5 C
Lacombe	Sp_Sp	26.2 A	40.6 A	1.4 B	2.3 C
	Sp	16.0 BC	29.2 AB	1.3 B	1.3 C
	Sp_St	23.2 AB	38.8 AB	7.5 A	11.6 A
	St	15.7 BC	26.9 B	4.8 AB	7.9 AB
	Fg	7.8 C	14.5 C	2.3 B	2.6 BC

* The inoculation treatments Sp_Sp, Sp, Sp_St, St, NI, and Fg represent double , and single applications of a spray inoculum, spread of a mixture of infested straw and spray of the same mixture of spore suspension, spread of infested straw only, uninoculated, and sprayed with the fungicide propiconazole, respectively.
‡ Means with the same letter are not significantly different according to Tukey's HSD at $P< 0.05$.

Table 2-10. Pearson's coefficients of correlation between the percentage leaf area diseased (%LAD) and disease incidence (%I) of scald and net blotch in Edmonton (above the diagonal) and Lacombe (below the diagonal).

Disease		Scald		Net blotch	
	Triat‡	%LAD	%I	%LAD	%I
Sclad	%LAD		0.95**	-0.30	-0.26
	%I	0.96**		-0.29	-0.21
Net blotch	%LAD	-0.29	-0.27		0.92**
	%I	-0.32	-0.28	0.98**	

‡ %LAD and %I represent the percentages leaf area scalded and disease incidence.
** Significant at $P < 0.01$.

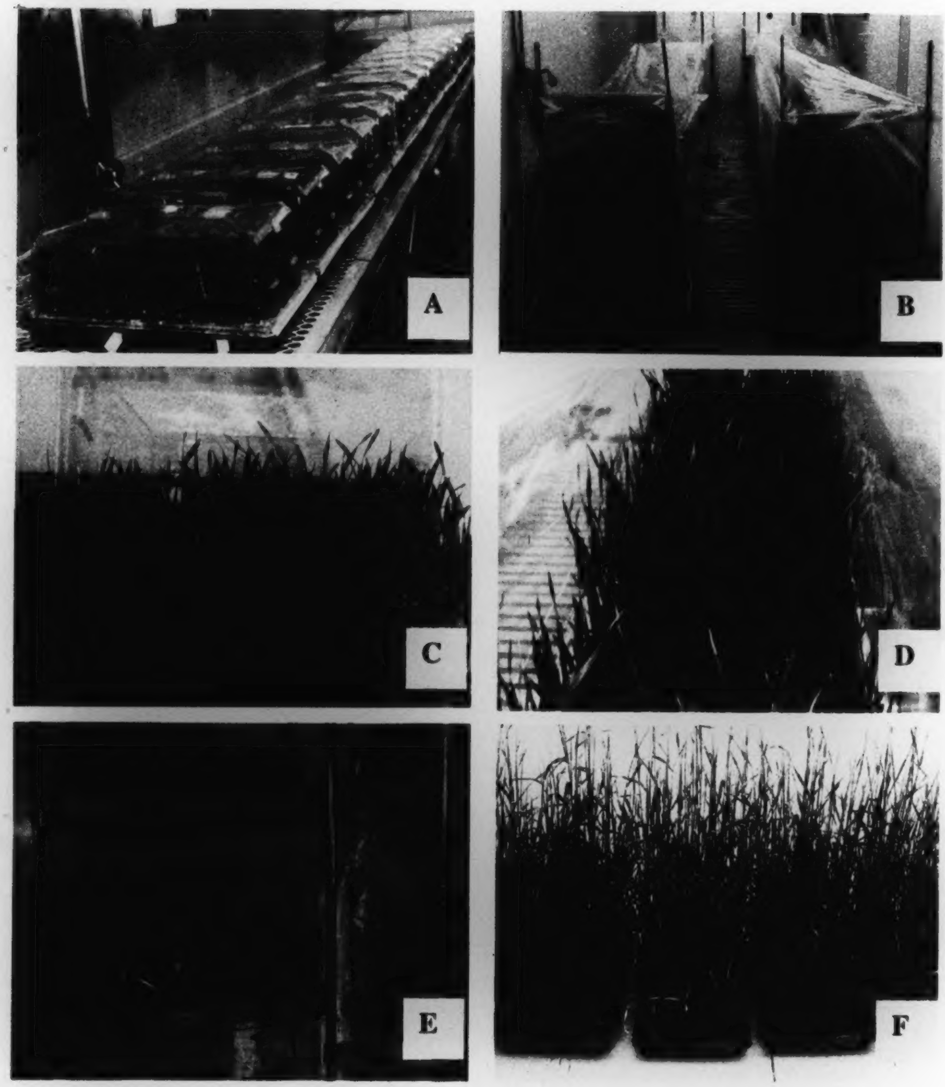

Figure 2-1. Indoor seedling and adult plant tests set-up. A: Seeding trays in the greenhouse with transparent plastic covers, B: Tent construction for adult plant test, C: Seedlings after inoculation at ZGS 12, D: Plants after inoculation (ZGS 32) in the adult plant test, E: Seedling trays before rating in the growth cabinet (ZGS 34) and F: Adult plants at ZGS 69.

Figure 2-2. Scald lesions on barley. Squares show areas from where successful isolation of scald was taken.

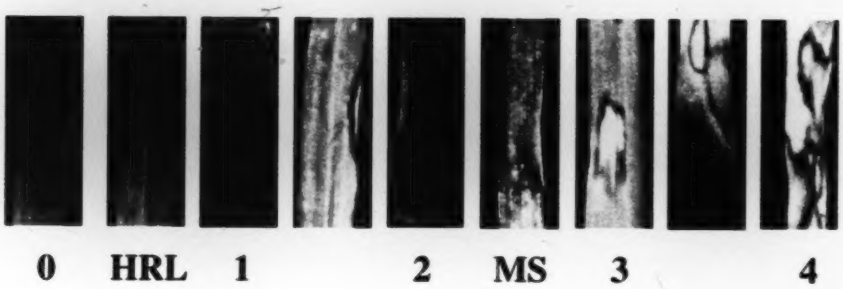

Figure 2-3. Scald infection response rating scale of 0-4 where 0, 1, 2, 3 and 4 are considered indicative of immune, resistant, moderately resistant, susceptible, and very susceptible seedling reactions, respectively. HRL and MS represent hypersensitive response-like (score 1) and moderately susceptible (score 2-3) infection types.

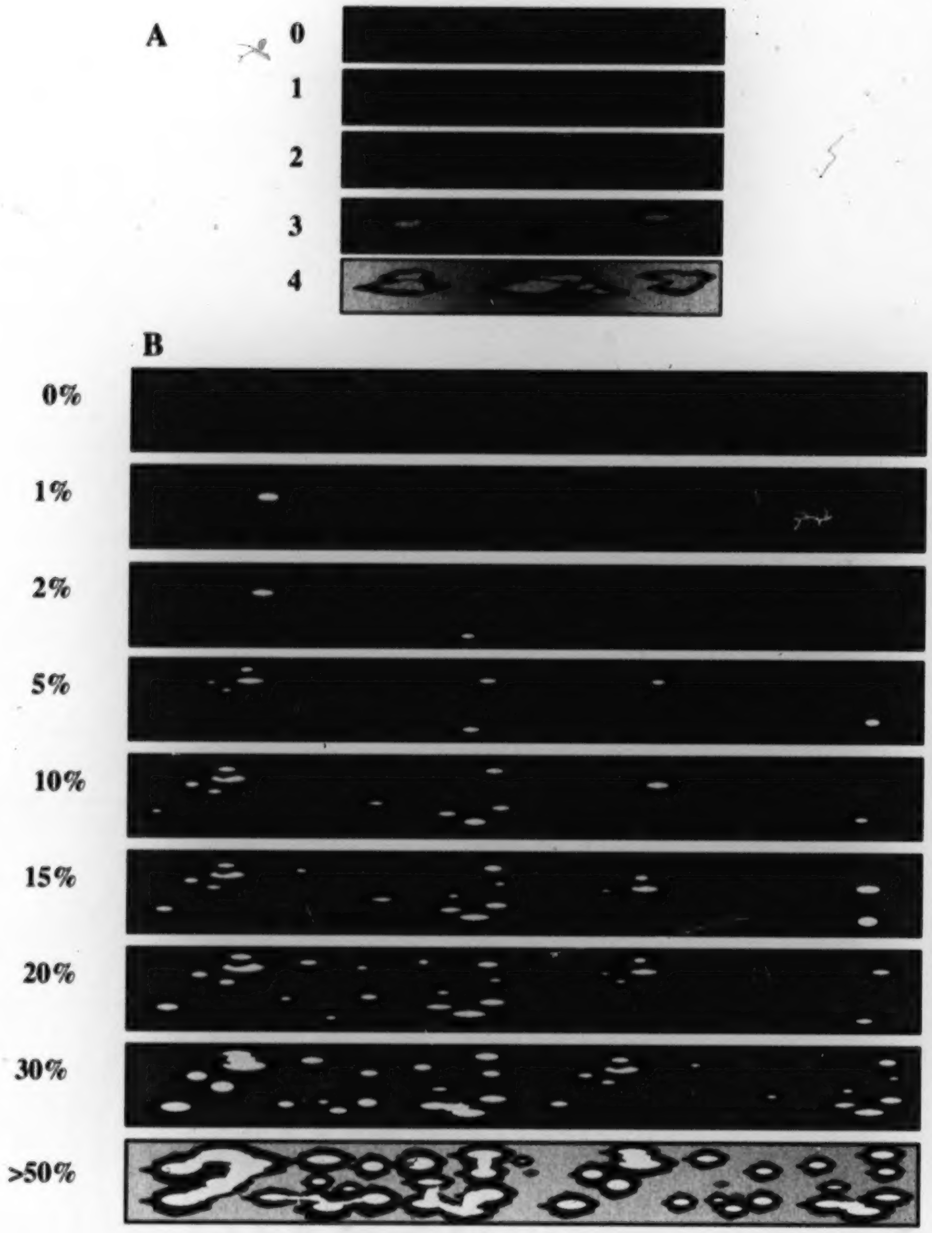

Figure 2-4. Scald scoring diagrams. (A) Types of disease reaction on seedlings. (B) Severity based on percentage area diseased. The percentage of disease symptoms in each section of diagram B was validated by scanning the pictorial area.

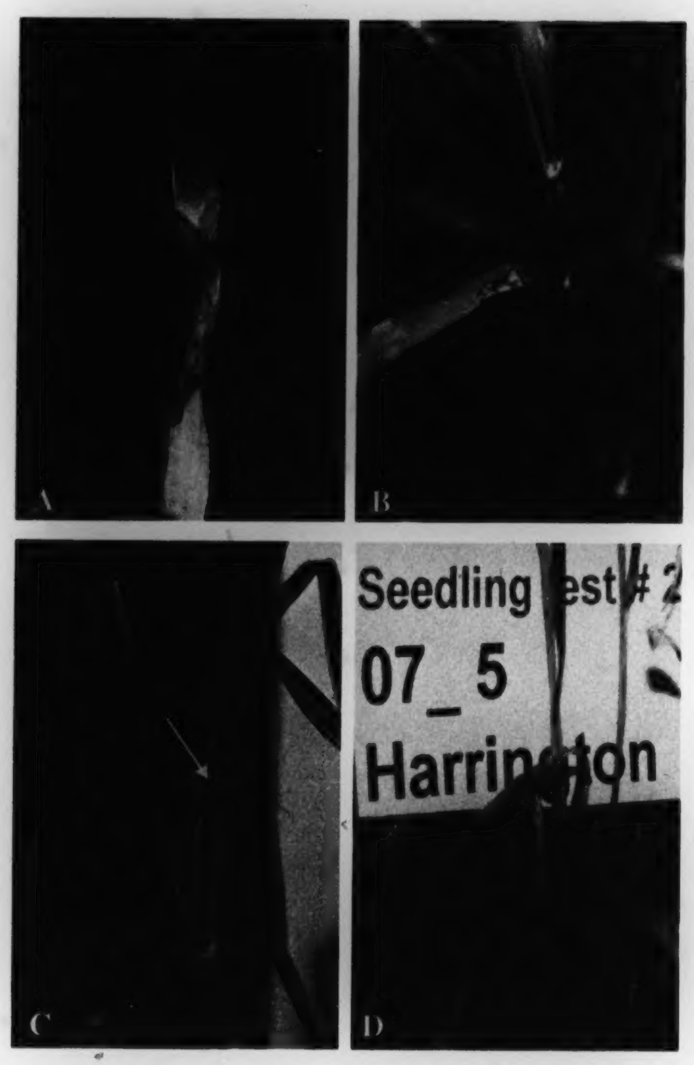
A
B
Seedling test # 2
07_5
Harrington
C
D

Figure 2-5. Scald symptoms at the seedling stage. A: An atypical lesion of scald at a partially wilted second leaf of barley seedling, B: Overlapping typical scald lesions, C: A grayish green lesion of scald at the leaf margin without typical brownish margins and D: Wilted leaves (inoculated at Zadoks' growth stage 12).

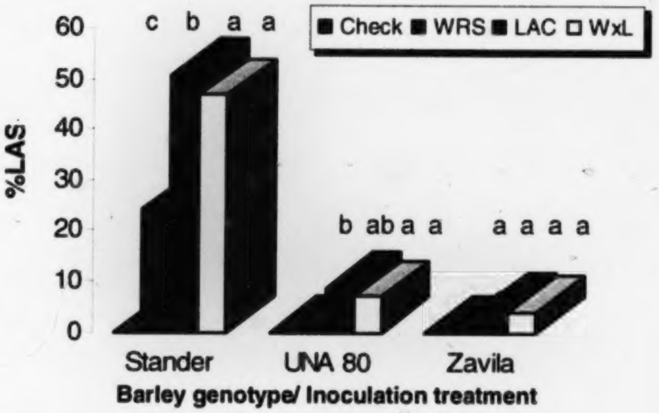

Figure 2-6. Bar graph presentation of the differences among inoculation treatments within barley genotypes studied in Case Study 1. Check, WRS, LAC, and W x L represent uninoculated and inoculated with isolate WRS 1860, Lac-01, and a mixture of both isolates, respectively. %LAS represents the percentage leaf area scalded. Treatments with same letter are not significantly different according to Tukey's pair-wise comparisons test (HSD) at P< 0.05.

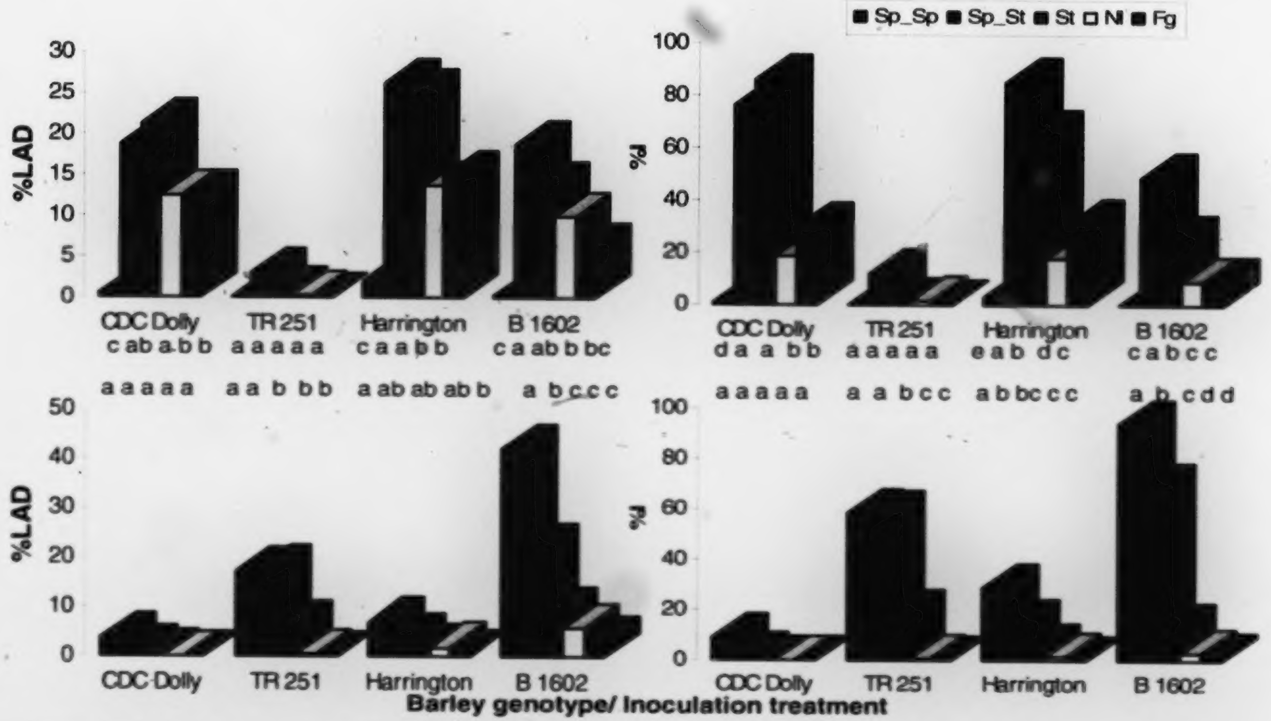

Figure 2-7. Bar graph presentation of differences among inoculation treatments within barley genotypes studied in Case Study 2 in Edmonton. The inoculation treatments Sp_Sp, Sp_St, St, NI, and Fg represent double applications of a spray inoculum, spread of a mixture of infested straw and spray, of the same mixture of spore suspension, spread of infested straw only, uninoculated, and sprayed with the fungicide propiconazole, respectively. %LAD and %I represent the percentages leaf area diseased and disease incidence, respectively in lower (scald) and upper (net blotch) graphs. Treatments with same letter are not significantly different (Tukey's pair-wise comparisons test (HSD) at $P< 0.05$).

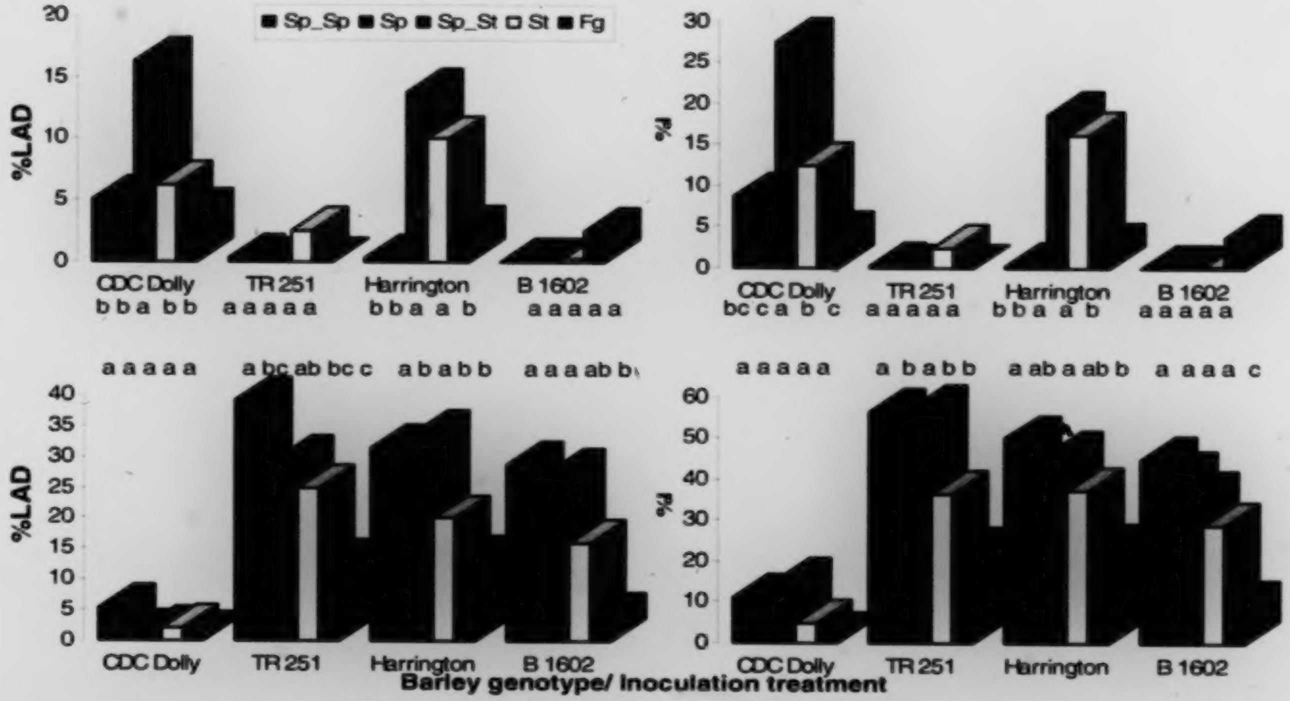

Figure 2-8. Bar graph presentation of differences among inoculation treatments within barley genotypes studied in Case Study 2 in Lacombe. The inoculation treatments Sp_Sp, Sp, Sp_St, St, and Fg represent double and single applications of the spray inocula, spread of a mixture of infested straw, and spray of the same mixture of spore suspension, spread of infested straw only, and sprayed with the fungicide propiconazole, respectively. %LAD and %I represent the percentages leaf area diseased and disease incidence, respectively in lower (scald) and upper (net blotch) graphs. Treatments with same letter are not significantly different (Tukey's pair-wise comparisons test (HSD) at $P< 0.05$).

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Chapter 3

Slow-scalding resistance in western Canadian barley cultivars[§]

3.1. Introduction

Various types of scald resistance genes have been identified in cultivated barley, *Hordeum vulgare* L., and its relatives (Jörgensen, 1992). As for other disease resistance genes, resistance to *R. secalis* can be classified into race-specific and non race-specific. Major genes conferring race-specific resistance to scald have been extensively used into barley breeding programs, but due to extreme variability and the emergence of new pathogenic races of *R. secalis*, have turned out to be short-lived (Tekauz, 1991). In contrast, the non race-specific resistance is theoretically considered to be governed by a number of minor genes, to operate equally against all pathogenic races, and hence to be more durable (Van der Plank, 1984). Although it is so far not shown in the barley-scald pathosystem, this type of resistance is considered to be a possible solution to the instability of race-specific resistance (Shaner, 1996). Historically, most cultivated barleys in Alberta have been susceptible to the scald disease (Anonymous, 1989-2004). Among the recommended barley varieties for Alberta during 1999-2004, only four cultivars (Seebe, Kasota, Mahigan, and Niska) were reported to remain resistant, whereas all malting barley cultivars registered in western Canada were susceptible to scald. Despite the fact that the Canadian breeders have made significant efforts with regard to the development of resistant cultivars, the scald resistance in a substantial number of these varieties has been breaking down, especially in Alberta (Xi *et al.*, 2003). Hence, there is a need to identify new/existing sources of resistance and examine their effectiveness while interacting with mixtures of pathogen isolates in different environments.

[§] A part of this chapter has been published in:

Sorkhilalehloo, B., J.P. Tewari, T.K. Turkington, F. Capettini, K.G. Briggs, B. Rossnagel, and R.P. Singh. 2000. Slow-scalding in some western Canadian barley cultivars. (Abstr.) International Symposium of Durable Disease Resistance, Ede-Wageningen, The Netherlands, Nov. 28- Dec. 1, 2000, p. 91.

Sorkhilalehloo, B., J.P. Tewari, T.K. Turkington, F. Capettini, K.G. Briggs, B. Rossnagel, and R.P. Singh. 2001. Slow-scalding in barley, a novel strategy for disease management (Abstr.). Can. J. Plant Pathol. 23:190.

Slow-scalding resistance (S-SR), the phenomenon of slow disease development under severe epidemic, is a relatively newly-discovered phenomenon in the barley-scald pathosystem, which due to the instability of major gene type of resistance has been gaining increasing attention (Ginkel and Vivar, 1986). As in adult plant resistance in the wheat-rust pathosystem, (Singh and Huerta-Espino, 2003), slow-scalding can be characterized as a combination of susceptible reaction at the seedling stage and adequate level of field resistance at the adult plant stage. For example, the cultivar Leduc was found to be susceptible to the majority of scald isolates tested by Tekauz (1991), still showing a good level of adult-plant resistance under field conditions (Xi *et al.*, 2003; Xue *et al.*, 1995). Slow-scalding lines like those which have been used in this study (UNA 80 and Zavila) can be recognized by their low to intermediate levels of disease progress characteristics over the years (Ginkel and Vivar, 1986). Area under the disease progress curve (AUDPC) and apparent infection rate (r) can be appropriate tools for identifying this type of resistance. AUDPC and r measure different aspects of resistance and can result in greater discrimination between genotypes (Singh and Rao, 1989). A standardized AUDPC (SAUDPC) could facilitate making comparisons among epidemics of different durations (Campbell and Madden, 1990).

This study was undertaken to study quantitative reactions of many western Canadian barley cultivars (WCBC) to mixtures of scald isolates under field conditions at Edmonton and Lacombe, Canada, and Toluca, Mexico. A set of barley scald differentials with known resistance gene(s) to *R. secalis* and two ICARDA (International Center for Agricultural Research in the Dry Areas) / CIMMYT (International Maize and Wheat Improvement Center) slow-scalding genotypes were also included in the test to compare their field reactions with the Canadian barleys, and to determine the presence of S-SR in WCBC.

3.2. Materials and Methods

3.2.1. Field locations/years

The western Canadian barley cultivar test (WCBCT) was conducted at the University of Alberta Research Station, Edmonton (53°/33'N latitude, 113°/28'W longitude and 668 m elevation), Canada for 3 consecutive years (1999-2001). The experiment was also carried out both at the Agriculture and Agri-Food Canada, Lacombe Research Center (52°/28'N latitude, 113°/44'W longitude and 853 m elevation), Canada, and the CIMMYT's high rainfall research station at Toluca (19°/24'N latitude, 99°/12'W longitude and 2640 m elevation), Mexico, during 2000 and 2001.

3.2.2. Plant materials

The plant materials included 38 WCBC recommended for cultivation in Alberta (Anonymous, 1999), local resistant cultivars (cvs. Seebe and Kasota for Edmonton and Lacombe, respectively; and cv. Shyri for Toluca), a core set of 9 differentials with known genes for resistance (Tekauz, 1995), and two ICARDA/CIMMYT slow-scalding lines i.e. UNA 80 and Zavila (Ginkel and Vivar, 1986).

3.2.3. Inoculation

In this study, a combination of different techniques was used to artificially inoculate plants under field conditions (Chapter 2). At Lacombe and Edmonton, both the spreading of infested straw and spraying of a mixture of 6 different single-spore isolates of the scald fungus were used during the early stages of stem elongation at Zadoks' growth stages (ZGS) of 31-33 (Zadoks *et al.*, 1974). The infested straw had been collected from the scald nurseries of the previous year at the Lacombe Research Station. A handful of chopped straw was spread on a hill of 8-10 plants. Hill plots were 0.5m apart. The spray-inoculum consisted of the Canadian isolates of *R. secalis* listed in Table 2-3 of Chapter 2 (09-7, 07-5, B11-4, WRS 1860, Lac-01, and 27-11). The final spore density of each component was adjusted to 1.0 x 10^5 conidia per ml, and 0.1% Tween 20 was added into the solution. Approximately 5 ml of the mixture of all 6 isolates was uniformly sprayed over plants in each hill. At Toluca, a different method of inoculation was used, where a crop mixture of different but fully susceptible (fast-scalding) barley genotypes was sprayed with inocula made using the isolated spores from the previous season, harvested freshly, and placed on plants at ZGS 33. A single plot consisted of two 1-meter rows of each barley line. This method has been part of the routine scald evaluation system at CIMMYT for many years.

3.2.4. Experimental design, traits, and statistical procedures

The plant materials were sown in an alpha lattice design with three replicates in each location/year. To measure disease progress, multiple disease assessments were undertaken using the five uppermost leaves, which were arbitrary grouped into one lower, two middle, and two upper representing lower (L), middle (M), and upper (U) canopy levels, respectively. All genotypes were visually evaluated for scald disease severity (the average percent of leaf area scalded, %S) and incidence (the percentage of tillers/plot having scald lesions, %I), several times until physiological maturity (ZGS 37-83). The amount of severity (%S) was calculated based on an arithmetic average of the values for %S using the following formula:

$$\%S = \{3*[(L+M)/2]+2*U\}/5$$

At the last rating (ZGS 83), disease severity was estimated as final severity (FS, 0-100) and terminal score (TS, 0-9). Standardized area under the disease progress curve (SAUDPC) and apparent infection rates (r) were calculated for the %WS and %I data (Campbell and Madden, 1990). The formula for SAUDPC was:

$$\text{SAUDPC} = [\sum_{i}^{n-1} (\frac{Y_i + Y_{i+1}}{2})(t_{i+1} - t_i)] / (t_n - t_1)$$

where n = the number of evaluation times; Y_i = scald intensity (severity/incidence) at *i*th evaluation day. As seen in the formula, the SAUDPC was calculated by dividing the AUDPC value by the total duration of the epidemic that is $(t_n - t_1)$. The numbers of ratings in Edmonton were 10 (ZGS 37, 41, 45, 49, 61, 65, 69, 73, 77, and 83), 6 (ZGS 37, 41, 49, 65, 73, and 83), and 5 (ZGS 37, 49, 65, 73, and 83) times for 1999, 2000, and 2001 summers, respectively, whereas WCBC were rated 3 times (ZGS 41, 65, and 83) in other locations/years. The plant materials at Lacombe were rated twice (ZGS 41 and 83) during the summer of 2001. As suggested by Vanderplank (1963), apparent infection rate was estimated by regressing the logit of disease proportion (Ln x/(1-x)) on times in days (t). In summary, the traits used in statistical analyses were: FS= Final %S; AS= SAUDPC for %S; rS= r for %S; FI= Final %I; AI= SAUDPC for %I; rI = r for %I; and TS. For the data expressed as percentages, inverse sine transformation was applied (Steel *et al.*, 1997). Based on both separate and combined analyses of field data, the WCBC were studied for their degrees of slow-scalding resistance to *R. secalis*. To combine data from different environments, the homogeneity of error variances was tested using Bartlett's test (Steel *et al.*, 1997). All the aforesaid traits were analyzed with Proc Mixed, Proc Corr, Proc GLM, Proc Factor, or Proc Cluster using SAS (SAS Institute Inc., 1989).

3.3. Results

Overall conditions for development of the scald disease in all test environments (locations/years) were favorable, and artificial inoculation in all locations/years resulted in relatively uniform epidemics suitable for multiple disease assessments.

Table 3-1 presents analyses of variance of the traits studied for all test environments. It also shows the significance of heterogeneity of the error variances of tested environments. Significant differences were observed among WCBC for all traits in all locations/years except at Lacombe during 2001, where rS and rI were not statistically different among the genotypes. Combined analyses of variance were performed for data from the environments with homogeneous error variances (Table 3-2). Significance was observed among different environments (Env), genotypes (Gen) and their interaction (Gen x Env) for all traits. The data from Canada and Mexico were not pooled even if they had homogenous error variances.

Tables 3-3, 3-4, and 3-5 show the least square means (Ls-means) of barley lines/cultivars studied at Edmonton, Lacombe and Toluca, respectively. In each location, levels of resistance/susceptibility as determined by the intensity (severity, FS and TS, and incidence, FI) of disease varied among the genotypes. For example, genotypes with terminal severity of ≤ 4, ≥ 5 and ≤ 7, and ≥ 8 were observed at Edmonton (CDC Dolly, CDC Dawn, and Foster), Lacombe (Zavila, Stander, CDC Sisler), and Toluca (AC Harper, Tukwa, and CDC Fleet), respectively. Additionally, the amount (AS and AI) and the rate (rS and rI) of scald progress were different between and within genotypes with similar resistant, intermediate, and susceptible reactions to *R. secalis*. CDC Dolly showed adequate level of quantitative resistance to *R. secalis* among the WCBC. A genotype with a high level of resistance at one location did not necessarily perform in a similar way at other locations. CDC Dolly and AC Harper were good examples of genotypes and their resistance genes were not effective in all locations. The most susceptible genotype at one location also did not receive the lowest rank at the other locations. As determined by terminal disease severity, Foster (TS > 8) was the most susceptible genotype at Edmonton whereas its score at Lacombe and Toluca was about 7. There were genotypes which had similar trends for scald progress in all locations (resistant, Osiris; intermediate, AC Lacombe; and susceptible, CDC Sisler). To statistically compare the reactions of barley genotypes and to differentiate among their quantitative resistance to scald with respect to the disease progress, a Tukey's pair wise multiple means test was performed on the Ls-means (SAS Institute Inc., 1989). The results are shown in Table A-3 to A-6. For the sake of simplicity and abbreviation, only significance levels were tabulated for the pair wise comparisons, where an order number (Ent, as assigned in Table 3-3 to 3-5) was used to indicate the cultivars/line name. In each table, two sets of comparisons were included to compare genotypes for a given trait in both Canada vs. Mexico, or different traits in the same country (e.g. AS vs. AI and rS vs. rI).

In Figure 3-1, diseases progress of the cvs., CDC Earl and Leduc are shown at Edmonton in 2001. The pair wise comparisons between these cultivars indicated significant differences ($P< 0.01$) for their disease intensity. While the cultivar Leduc exhibited slow-disease development (AS=6.1%) and maintained an adequate level of field resistance (FS= 9.4%) during the course of epidemics, the severity of disease on CDC Earl (FS= 49.8%), demonstrated a high level of progress (AS=61.1%). Similarly, as disease was progressing in such a fast-scalding genotype, %LAS in all canopy levels increased. As illustrated in Figure 3-1A for CDC Earl, the contribution of flag leaf (FL) and the penultimate leaf (FL-1) in %LAS was higher than that of lower and middle canopy levels towards last two ratings. This was considered important since the last two ratings fell into the post-anthesis stages of barley plant and any losses of the photosynthetic areas of FL and FL-1 could directly contribute towards yield loss. In contrast, the slow-scalding genotype, Leduc, not only had less %LAS on their FL and FL-1, but also the portion of disease on their upper leaves was less than those of the middle and lower canopy levels. The results also revealed that in addition to disease severity, disease incidence in slow-scalding lines showed a low rate of progress compared to those of fast-scalding lines (Leduc: FI = 12.9%, AI= 13.2%; CDC Dolly: FI = 14.9%, AI= 20.5%; UNA 80: FI = 27.7%, AI= 20.1%; CDC Earl: FI = 82.3%, AI= 79.2%).

Figure 3-2 shows the AUDPC and terminal severity (TS, 0-9) of the differentials used in this study at different locations. Arbitrary selection points of 20 and 4 were set (red lines) for AUDPC and TS, respectively, to indicate the levels around which slow-scalding resistance could be looked for. It seemed that the field reaction and quantitative amount of disease resistance depicted by TS and AUDPC were different among the differentials carrying major-type of resistance. Both traits demonstrated similar differentiation among the differentials. However, there were examples where differences among differentials in terms of one trait were not necessarily determined by the other trait. With reference to this discrepancy, La Mesita and Trebi received similar TS at Lacombe whereas the value of AUDPC for La Mesita (42.8) was significantly higher than that of Trebi (24.1). Additionally, as shown in Figure 3-2, the disease levels were different both among locations and differentials. In Canada, the disease level was higher at Lacombe. However, approximately the same patterns of resistance were observed at Edmonton and Lacombe in contrast to Toluca where a different virulence spectrum of the pathogen was expected. More importantly, the scald resistance in Atlas and Atlas 46, proposed to have Rh2 and Rh3 genes (Dyck and Schaller, 1961), was shown to be quite effective in Canada whereas at Toluca those genes have been overcome. In contrast, La Mesita, which was resistant at Toluca, showed a relatively high amount of disease progress under Lacombe conditions.

On the other hand, the present study also indicated that Modoc and Trebi were intermediate in reaction to scald. Resistance in Osiris, looked stable and promising. Osiris with Rh4 gene showed TS of 3.4 or less during all different years and locations. Similar reactions compared to those of differentials were observed among the WCBC (Figure 3-3). The cultivars shown in this figure, except for Jackson which was susceptible across all locations/years, showed low to intermediate level of disease progress. The cultivars Zavila (an ICARDA/CIMMYT slow-scalding check) and CDC Dolly were the most resistant genotypes tested in Canada. AC Harper which was classified as a cultivar with intermediate reaction to scald at Lacombe showed a very resistant reaction in Toluca where the most effective Canadian sources of resistance reacted as susceptible genotypes. Interestingly, the cultivar CDC Dolly, which was considered as a slow-scalding line in Canada for several years, was fully susceptible at Toluca. Similar reactions were observed for the cultivars, Seebe, Mahigan, and Kasota, which are being used as resistant cultivars in Canada (Dr. F. Capettini, Head, ICARDA/CIMMYT Barley Breeding Program, personal communication). The slow-scalding check, UNA 80, performed at Toluca as it had done during the last 18 years. It was identified as a slow-scalding genotype in Canada as determined by its final severity at Edmonton and Lacombe. However, disease progress on this genotype was intermediate as compared with those of Zavila and Jackson which were low and high, respectively. The cultivars Leduc, Stetson, Phoenix, and AC Lacombe all had intermediate levels of disease progress and a disease score of <6.

The reaction of all genotypes tested were further classified using hierarchical cluster analysis of their FS, FI, AS, AI, and TS in Edmonton, Lacombe, and Toluca, where Seebe, Kasota, and Shyri were included as a local resistant check at each location, respectively (Figure 3-4, 3-5 and 3-6). Using all aspects of disease development (severity, incidence, rate, and amount of progress), cluster analyses resulted in classification of the genotypes studied into different resistance groups at each location studied. An arbitrary cut-off point (red lines in the figures) was selected at the distance of 0.50 to classify the genotypes at the corresponding similarity level. This resulted in grouping the most susceptible (Edmonton, Foster; Lacombe, Jackson) and one of the most resistant (Edmonton, Seebe; Lacombe, Atlas 46; Toluca, Shyri) genotypes into separate one-entry clusters in all locations except for Toluca where the most susceptible entry (CDC Down) was grouped in a multiple-entry cluster. In all dendrograms, the genotypes were classified into resistant (R), slow-scalder (S-S), intermediate (I), and susceptible/fast-scalder (S) categories. As shown in Figure 3-4 at Edmonton, the cluster of cultivars Zavila, Osiris, and CDC Dolly was the closest resistant group next to that for Seebe, the resistant local check. The slow-scalding genotypes, the majority of differentials studied, and the WCBC with intermediate levels of disease progress all were located into three neighboring clusters. Exception was Brier (D) which as differential was recognized as susceptible as Harrington in the susceptible cluster. At Lacombe (Figure 3-5), the differential, Atlas 46 alone made a resistant cluster and cultivars like Zavila, CDC Dolly and Kasota were grouped into the R/S-S cluster representing the majority of differentials studied. Brier (D) and Modoc fell into the susceptible category whereas La Mesita and a set of slow-scalding and moderately resistant/susceptible genotypes made another group (Cluster I). In Toluca (as shown in Figure 3-6), La Mesita, Modoc, Trebi, and Osiris as differential lines were grouped with UNA 80, AC Harper, and AC Hawkeye, and together with Shyri and another resistant group including Zavila, Bronco and Brier, made the R/S-S cluster. The intermediate group consisted of 3 differentials which were grouped with cvs. Leduc, Stetson, AC Lacombe, and Phoenix. Atlas and Atlas 46 were fully resistant but were classified with the most susceptible genotypes in Toluca.

Table 3-6 presents the results of factor analysis on the WCBC for the data obtained from all locations/years. The results showed that all the studied traits were independent variables since the analysis resulted in seven different PCs. The first two PCs accounted for a total of 93.64 of the total variation making them a useful means for the classification of observations. The component matrix of the PCs also showed that the PC2 accounting for 23.89% of the total variation were highly related to the rate of disease progress (rS and rI) in contrast to the first component, which included all traits related to the amount of disease. This fact was illustrated using a component plot shown in Figure 3-7. It was suggested that the amount and rate of disease progress were of two different types and both must be considered important. The linear relationship and significance of all recorded/computed traits, which were measured using Pearson correlation coefficients, are given in Table 3-7. The results showed that the values of AUDPC and r are not necessarily correlated. The result of correlation analysis was in accordance with that of the factor analysis.

3.4. Discussion

Genotypic and environmental variation

In order to determine presence or absence of genotypes with S-SR, quantitative reactions of all the genotypes were studied in different locations/years. Analysis of variance with the data of each location/year showed significant differences among the barley genotypes studied. There was an indication of differences in quantitative reactions of the genotypes for the trait demonstrating severity and incidence of the scald disease. However, there were no significant differences for rS and rI at Lacombe during 2001. This could be due to less number of assessments for the experiment carried out at

Lacombe during that year. Apparent infection rate could be more reliable and differentiating once calculated based on more ratings for both disease severity and incidence.

Environmental conditions at Edmonton, Lacombe, and Toluca are usually considered favorable for the development of barley scald (Ginkel and Vivar, 1986; Turkington *et al.*, 1998). However, analysis of variance was not attempted to combine the data over Canada and Mexico since the components of artificial inocula applied at Toluca were unknown and different. In contrast, data from Edmonton and Lacombe were could be combined/analyzed since the same artificial spray-inocula were applied at the locations/years studied within Canada. It seemed that the level of disease at Edmonton was slightly lower than that observed under Lacombe conditions. The differences observed among the Env and Gen x Env sources of variation could be due to the effects of environmental parameters, differences among pathogenic variation in *R. secalis* isolates' populations at different locations, their interactions with the studied genotypes, and how each environment may affect both the fungus and the host separately and/or their interaction together. In this experiment, natural precipitation was relied upon to provide the necessary humidity for infection and disease spread. However, at Edmonton during the hot days, overhead irrigation was applied to maintain optimum conditions for disease progress.

In practice, slow-scalding lines are expected to demonstrate adequate field resistance during the course of epidemics (Dr. B. G. Rossnagel, Crop Development Centre, Saskatoon, Saskatchewan, personal communication). In this study, slow-scalding resistance (S-SR) was considered synonymous with quantitative resistance as genotypes with this type of resistance exhibited a compatible reaction with *R. secalis* coupled with low to intermediate levels of disease progress (with an AS close to 20%) under favorable conditions for disease development at the adult-plant stage. We arbitrarily selected As = 20% and TS = 4 to set a cut-off point for studying S-SR. However, statistically disease progress levels which are not significantly different from those of slow-scalding lines could also be considered low to intermediate. Hence, genotypes with $2 < TS < 5$ are should be retained if the ultimate goal is to breed for S-SR. Since two genotypes with the same TS could show different levels of disease intensity and progress during the course of an epidemic, taking the rate and amount of disease progress for severity and incidence data into account, would result in a more effective selection among genotypes with the same score. Within a given TS class, a lower AS might be an indication of lower rate of progress or late-scalding.

G x E interactions

It was shown that the slow-scalding genotypes do not necessarily perform in a similar way in different locations/years. This indicated that the genes conditioning S-SR do not equally operate against all pathogenic races and hence may not be durable based on the absolute concept of durability described by Johnson (1993). However, it does not mean that the performance of slow-scalding lines over limited locations/years cannot be practically stable, as was observed for ICARDA/CIMMYT slow-scalding lines and some Canadian genotypes resistance to local pathotypes of *R. secalis*. In contrast to the term durability, stability can be applied to any resistance level. Therefore, a barley genotype challenged with the scald pathogen under field conditions may show a score of 5-6 out of 9 over many locations/years. For example, AC Lacombe, AC Hawkeye, Leduc, Brier, CDC Harper, and Falcon were consistently rated as cultivars showing intermediate type reactions to scald. Interestingly, cvs. Leduc and Brier, first registered as resistant, due to emergence of pathotypes which matched their virulence genes to those of the resistance in plants, were later re-claimed to have an intermediate type of reaction, few years after their release (Anonymous, 1989-2004). The stability at a given resistance level might reflect an equilibrium among factors affecting disease resistance including resistance genes in the host (G_h), virulence genes of local populations of the pathogen and their aggressiveness (G_i), environmental conditions (E), and all different kind of interactions (G_h x E, G_i x E, and G_h x G_i x E) at that level. Hence, changes in these factors could result in changes in plant responses to the pathogen. Transient responses of a plant to biotic stresses are usually due to instability of resistance conditioned by major genes. In Alberta, resistance to *R. secalis* in CDC Fleet, CDC Earl, CDC Guardian, Duke, and CDC Dawn are examples of this type of transient resistance (Anonymous, 1989-2004). Deployment of effective and more stable resistance genes while placing less selection pressures on the pathogen populations, on the other hand, may assist breeders/pathologists in managing the scald disease in a more sustainable way.

Implications from resistant checks and barley scald differentials

A comparison between a local slow-scalding check and a genotype of interest could also provide us with a fast means of screening materials for slow-scalding. The present study revealed that the ICARDA/CIMMYT slow-scalding lines, Zavila and UNA 80, could be considered as useful checks in Canada and Mexico. CDC Dolly, on the other hand, a good slow-scalder in Canada, was a weak performer in Mexico. In addition, other genotypes with a fairly stable resistance over both countries like Leduc, Stetson, Phoenix, and AC Lacombe looked promising for both being used as slow-scalding checks or to be used as desirable parents in crossing programs aimed at improving slow-scalding resistance.

The differential cultivars can be grouped to provide meaningful genetic information e.g. cultivars with related resistance genes can be grouped in the same category as a given differential (Goodwin *et al.*, 1990). In this study, the reactions of the genotypes were compared with those from differentials with known major genes for scald. The purpose of choosing differentials was to maintain continuity with the previous literature and to include some of the most important genes for scald resistance in this study. A study on several commercial barley cultivars and scald differentials at various locations in Alberta showed that Rh, Rh2, and Rh9 resistance genes could be used as effective sources of resistance in western Canadian barley breeding programs (Xi, 1999). Our results indicated that differentials with major genes could show different quantitative performance to scald depending on the environment used to assess their field reactions. It was shown that resistance genes (Rh2 and Rh3) in Atlas and Atlas 46 were only effective in Canada. In contrast the Rh gene in Brier was found not to be as effective as its allelic form Rh^3 in the differential, WW x G which was quite effective in all test environments. Osiris with the Rh4 gene also showed a TS of 3.4 or less during all years and locations. Trebi also has been reported to carry Rh4 (Dyck and Schaller, 1961) and demonstrated a stable and adequate level of resistance across all locations/years. Hence, the Rh4 gene could be considered to play an important role in stability/durability of resistance in all studied environments. However, adult-plant responses of differentials carrying Rh4 (Osiris, La Mesita, and Modoc) were not similar suggesting that there were differences among differentials in terms of their comparative reactions to *R. secalis*. These discrepancies might be due to the effects of modifying genes, different genetic backgrounds, or even different alleles at the same locus. Interestingly, scald progress on some of the differentials studied such as WW x G and Osiris indicated that they were slow-scalding as well. It may be inferred that a combination of both major and minor genes condition S-SR. Incomplete expression of a major gene could also contribute to S-SR. In practice, one may simply select for the slow-scalding type of resistance with an acceptable level of field resistance, even if it is controlled by a single gene. Hence, breeders could select among single major genes with higher stability over different locations/years. In this respect, the resistance in Osiris looked quite stable and promising, confirming results of previous workers (Ceoloni, 1980; Salamati and Tronsmo, 1997; Xi *et al.*, 2002).

Classification of barley genotypes

To get a clear picture of cultivar reactions across various locations, to summarize different aspects of disease progress, and to classify genotypes studied into categories, a combination of factor, correlation, and cluster analyses were further used. The factor analysis was intended to provide information both on the nature of the relationships between the studied traits, and to categorize the traits into possible independent principal components (PCs) which could account for different aspects of disease progression. It seemed difficult to explain how the traits explaining the variation due to second component were not associated with those explained by the traits in the PC1 since both disease progress and rate were expected to be highly associated. This may not confirm that disease progress/epidemic in the case of scald follows a logistic model of disease progression. Hence, experimental error might explain part of the discrepancies observed.

Using cluster analysis, genotypes with similarity in their characteristics related to disease intensity and progress were classified into different clusters representing various levels of resistance. Without such statistical tools and with respect to including various disease traits into a study, it would be difficult to precisely differentiate between a large number of genotypes with quantitative reactions and to classify them into resistant (R), slow-scalder (S-S), intermediate (I) and susceptible/fast-scalder (S) categories. It was also difficult to separate clusters or genotypes with similar reactions into different categories. However, by comparing the relative distance of a given genotype with its neighboring clusters where known differentials, slow-scalding, or susceptible checks were located, one could hypothesize whether the disease progress in that genotype was slow, intermediate, or fast. Such an approach might facilitate breeders/pathologist selection methodology in choosing both resistant and slow-scalding parents for their breeding programs. In this study, ICARDA/CIMMYT checks were also shown to be useful resources for slow-scalding resistance. Incorporation of resistance genes from ICARDA/CIMMYT into Canadian resistant cultivar development programs should be considered. According to the Field Crop Development Centre (FCDC) of Alberta Agriculture, Food and Rural development (AAFRD), that the resistant cultivars in western Canada such Seebe, Kasota, Mahigan, and Niska owe a great proportion of their resistance genetics to ICARDA/CIMMYT disease resistance barley germplasm (Helm *et al.*, 2001). Accumulating S-SR genes into genotypes acceptable for yield and quality purposes would help breeders to address problems regarding to instability of resistance to scald.

3.5. Tables and Figures

Table 3-1. Analyses of variance of the studied traits for western Canadian barley cultivars in all locations/years.

			Trait‡						
Location/ Year	S.o.V†	df	FS	rS	AS	FI	AI	rI	TS
Edmonton 1999	Rep	2	4.76	0.0001	3.69	20.10	10.31	0.0002	0.05
	Block (Rep)	20	17.59	0.0001	7.01	30.66	14.41	0.0005	0.32
	Gen	49	8.42 **	7.79 **	10.23 **	8.54 **	11.29 **	7.69 **	8.49 **
	Error§	71	87.48 c	0.0007 ab	27.06 c	221.80 cd	53.58 b	0.0024 b	1.68 bc
Edmonton 2000	Rep	2	22.42	0.0002	4.57	44.74	36.80	0.0001	0.38
	Block (Rep)	20	6.40	<.0001	1.41	<.0001	2.44	<.0001	0.37
	Gen	49	7.00 **	5.11 **	16.04 **	7.93 **	12.38 **	2.69 **	14.72 **
	Error	71	56.10 bc	0.0007 a	7.60 a	136.02 bc	47.21 b	0.0038 b	1.37 bc
Edmonton 2001	Rep	2	3.27	<.0001	14.50	1.22	9.97	0.0001	0.07
	Block (Rep)	20	4.99	0.0001	11.67	2.17	12.49	<.0001	0.06
	Gen	49	10.83 **	3.27 **	7.79 **	10.28 **	7.28 **	3 **	14.73 **
	Error	71	79.96 c	0.0011 b	120.26 e	360.24 d	235.90 d	0.0012 a	1.23 bc
Lacombe 2000	Rep	2	0.40	<.0001	0.27	<.0001	0.04	<.0001	0.05
	Block (Rep)	20	1.16	<.0001	0.29	<.0001	1.33	<.0001	0.04
	Gen	49	24.83 **	3.81 **	25.96 **	7.16 **	12.28 **	2.55 **	12.10 **
	Error	71	10.50 a	0.0005 a	2.47 a	65.07 a	10.53 a	0.0040 b	0.94 b
Lacombe 2001	Rep	2	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Block (Rep)	20	0.85	<.0001	7.90	18.64	17.08	<.0001	<.0001
	Gen	49	1.86 **	1.23	1.39	2.66 **	1.74 *	1.25	2.55 **
	Error	71	81.71 c	0.0040 d	47.21 d	218.31 bcd	482.20 e	0.0031 b	1.72 c
Toluca 2000	Rep	2	<.0001	0.0001	0.80	<.0001	0.47	<.0001	<.0001
	Block (Rep)	20	<.0001	0.0004	<.0001	<.0001	<.0001	0.0012	<.0001
	Gen	49	48.54 **	3.94 **	49.46 **	37.05 **	27.53 **	1.81 *	47.23 **
	Error	71	39.56 b	0.0048 d	13.52 b	76.93 a	36.95 b	0.0139 c	0.52 a
Toluca 2001	Rep	2	5.63	0.0003	36.99	<.0001	48.06	0.0001	0.01
	Block (Rep)	20	11.88	0.0002	31.95	38.54	28.34	0.0007	0.10
	Gen	49	11.81 **	6.84 **	10.63 **	15.59 **	12.55 **	5.4 **	17.59 **
	Error	71	52.50 bc	0.0022 c	116.22 e	134.56 b	130.78 c	0.0033 b	0.58 a

† S.o.V: Source of variation, Rep: Replicate, Gen: Genotype.

‡ FS= Final %severity; AS= Standardized area under disease progress curve (SAUDPC) for %severity; rS= Apparent infection rate (r) for %severity; FI= Final %incidence; AI= SAUDPC for %incidence; rI = r for %incidence; and TS: Terminal Severity (0-9).

* and ** Significant at P< 0.05 and 0.01, respectively.

§ Within a column, error mean squares with the same letter are not significantly different according to Bartlett's test (Steel *et al.*, 1997).

Table 3-2. Combined analyses of variance of the studied traits for western Canadian barley cultivars in different locations/years (environment, Env) with homogeneous variances of error.

	Four environments‡				Three environments		
S.o.V†	df	FS_CA	TS_CA	rI_CA	df	rS_CA	FI_CA
Env	3	3271.0 **	176.6 **	2.980 **	2	0.596 **	147734.6 **
Rep (Env)	8	464.0	8.9	0.011	6	0.006	1409.3
Block (Env*Rep)	108	107.4	2.1	0.004	81	0.001	257.9
Gen	48	1154.3 **	40.7 **	0.008 **	48	0.004 **	2045.5 **
Gen x Env	144	237.6 **	3.5 **	0.011 **	96	0.002 **	434.8 **
Error	276	75.7	1.3	0.003	207	0.001	191.9

	Two environments				
S.o.V	df	AI_CA	AS_CA	TS_MX	FS_MX
Env	1	14806.4 **	2273.5 **	57.5 **	29322.1 **
Rep (Env)	4	1305.8	134.8	1.1	215.4
Block (Env*Rep)	54	80.3	8.7	0.7	64.1
Gen	48	821.5 **	124.0 **	22.7 **	1621.9 **
Gen x Env	48	126.3 **	27.8 **	3.1 **	239.1 **
Error	138	50.3	4.7	0.6	47.9

† S.o.V: Source of variation, Rep: Replicate, Gen: Genotype, Evn: Environment.

‡ FS= Final %severity; AS= Standardized area under disease progress curve (SAUDPC) for %severity; rS= Apparent infection rate (r) for %severity; FI= Final %incidence; AI= SAUDPC for %incidence; rI = r for %incidence; and TS: Terminal severity (0-9).

FS_CA: Combined for FS over Edmonton1999-2001 and Lacombe 2001.

TS_CA: Combined for TS over Edmonton1999-2001 and Lacombe 2000.

rI_CA: Combined for rI over Edmonton1999-2000 and Lacombe2000-2001.

rS_CA: Combined for rS over Edmonton1999-2000 and Lacombe2000.

FI_CA: Combined for FI over Edmonton1999,2001 and Lacombe2001.

AI_CA: Combined for AI over Edmonton1999-2000.

AS_CA: Combined for AS over Edmonton 2000 and Lacombe2000.

TS_MX: Combined for TS over Toluca 2000-2001.

FS_MX: Combined for FS over Toluca 2000-2001.

* and ** significant at P< 0.05 and 0.01, respectively.

Table 3-3. Least square means of barley cultivars/lines for all traits tested at Edmonton, 1999-2001.

		Trait‡									Trait						
Ent†	Cultivar/Line	FS	rS	AS	FI	AI	rI	TS	Ent	Cultivar/Line	FS	rS	AS	FI	AI	rI	TS
1	CDC Silky	21.4	0.067	28.5	52.8	50.4	0.098	6.9	26	CDC Stratus	32.2	0.100	26.2	50.2	30.9	0.081	6.2
2	Bridge	14.9	0.062	12.2	24.0	18.3	0.066	3.6	27	Tercel	33.9	0.091	36.2	65.5	56.0	0.119	7.0
3	CDC Guardian	39.9	0.131	46.8	62.9	46.8	0.141	7.7	28	Duel	40.9	0.093	53.4	75.2	66.3	0.130	8.4
4	Leduc	9.4	0.032	6.1	12.9	13.2	0.004	3.5	29	AC Albright	19.4	0.051	25.5	62.7	51.8	0.097	5.7
5	CDC Earl	49.8	0.097	61.1	82.3	79.2	0.122	8.2	30	Bronco	37.0	0.088	35.9	74.1	65.7	0.091	7.2
6	WW x G	5.6	0.015	4.7	16.1	16.6	0.117	2.1	31	B1602	33.5	0.088	46.9	66.2	66.4	0.118	7.5
7	Falcon	16.1	0.057	18.6	36.1	32.3	0.066	4.4	32	TR139	37.0	0.096	41.1	51.2	37.6	0.075	6.8
8	Stein	27.0	0.077	23.2	34.2	27.3	0.075	5.7	33	AC Lacombe	19.9	0.057	19.3	30.2	31.1	0.037	4.5
9	AC Oxbow	37.2	0.096	42.9	75.8	63.1	0.124	7.7	34	CDC Dolly	3.7	0.000	6.5	14.9	20.5	0.035	2.0
10	AC Bountiful	37.5	0.096	43.9	61.1	49.5	0.112	8.2	35	Harrington	26.3	0.085	33.6	50.8	41.0	0.104	5.7
11	B1215	35.6	0.110	35.6	67.6	48.9	0.121	7.4	36	Modoc	18.6	0.054	18.5	36.4	31.3	0.044	4.3
12	UNA80	16.3	0.059	15.8	27.7	20.1	0.067	4.7	37	AC Harper	12.2	0.067	10.1	20.9	23.9	0.080	3.2
13	AC Metcalfe	27.1	0.092	27.2	47.3	32.0	0.078	5.1	38	Trebi	15.3	0.061	17.0	39.4	31.9	0.074	4.2
14	TR145	22.7	0.080	22.1	34.1	31.6	0.100	6.1	39	Osiris	1.5	0.012	1.0	8.7	5.3	0.038	0.8
15	Atlas46	0.9	0.008	2.3	4.7	6.7	0.026	1.1	40	AC Hawkeye	17.8	0.067	13.2	29.9	22.6	0.065	4.4
16	Stetson	13.0	0.044	17.1	36.4	33.0	0.036	3.6	41	Brier	19.3	0.068	8.6	38.8	8.8	0.065	5.3
17	La Mesita	15.8	0.084	11.9	38.7	23.3	0.118	4.1	42	Duke	32.2	0.071	47.1	76.6	77.4	0.093	7.4
18	Jackson	44.1	0.098	66.6	74.4	73.5	0.112	8.2	43	CDC Gainer	31.7	0.115	26.7	49.9	38.1	0.143	6.7
19	Atlas	6.1	0.040	5.8	21.7	11.0	0.048	1.8	44	Zavila	4.9	0.017	1.2	5.6	1.2	0.020	0.7
20	Tukwa	12.8	0.041	16.5	27.2	21.8	0.032	3.0	45	AC Rosser	44.8	0.087	56.6	71.7	66.4	0.075	8.1
21	CDC Dawn	28.3	0.093	31.6	51.8	42.7	0.118	6.4	46	Stander	38.3	0.074	53.8	64.4	68.2	0.091	7.6
22	CDC Sisler	41.7	0.094	58.6	82.2	81.8	0.124	8.4	47	CDC Fleet	31.0	0.083	37.4	58.9	55.0	0.104	6.6
23	Turk	19.7	0.104	12.7	28.8	21.9	0.118	4.7	48	Foster	47.5	0.088	75.3	83.0	85.9	0.112	8.3
24	Phoenix	20.3	0.077	21.5	49.9	38.2	0.059	5.1	49	Brier (D)	25.6	0.048	26.5	41.4	37.6	0.026	5.3
25	Manley	22.9	0.076	20.9	41.0	21.5	0.046	4.7	50	Seebe	3.9	0.023	5.0	5.3	32.9	0.026	0.8

† Ent.: Entry. Numbers 1-50 represents cvs. CDC Silky to Seebe at Edmonton, respectively.

‡ FS= Final %severity; AS= Standardized area under disease progress curve (SAUDPC) for %severity; rS= Apparent infection rate (r) for %severity; FI= Final %incidence; AI= SAUDPC for %incidence; rI = r for %incidence; and TS: Terminal Severity (0-9).

Table 3-4. Least square means of barley cultivars/lines for all traits tested at Lacombe, 2000-2001.

		Trait‡					
Ent†	Cultivar/Line	FS	rS	AS	FI	AI	rI	TS
1	CDC Silky	30.2	0.155	39.9	93.4	77.3	0.158	6.1
2	Bridge	33.8	0.120	45.9	92.0	76.9	0.151	5.6
3	CDC Guardian	41.7	0.123	59.3	95.6	85.0	0.143	8.0
4	Leduc	25.0	0.114	32.2	90.2	71.4	0.162	5.4
5	CDC Earl	35.6	0.125	52.2	94.8	85.6	0.183	7.2
6	WW x G	19.0	0.102	24.7	65.1	54.8	0.088	3.9
7	Falcon	27.4	0.126	35.3	92.4	71.1	0.164	5.8
8	Stein	32.6	0.172	42.1	86.0	66.4	0.209	6.9
9	AC Oxbow	37.5	0.108	52.8	93.8	87.9	0.134	7.3
10	AC Bountiful	33.8	0.152	45.0	85.1	70.6	0.160	6.9
11	B1215	31.1	0.132	39.8	93.8	73.6	0.164	7.4
12	UNA80	30.6	0.112	42.5	93.0	80.1	0.157	4.9
13	AC Metcalfe	27.0	0.105	39.4	88.0	80.1	0.148	5.7
14	TR145	27.7	0.121	35.3	90.3	70.1	0.178	5.8
15	Atlas46	13.0	0.081	20.1	37.4	21.1	0.153	3.3
16	Stetson	25.0	0.115	33.5	93.7	71.3	0.170	5.4
17	La Mesita	26.8	0.091	42.8	87.7	78.3	0.156	4.6
18	Jackson	47.3	0.126	80.1	94.7	90.7	0.132	8.2
19	Atlas	13.2	0.106	11.7	58.0	42.7	0.099	3.2
20	Tukwa	31.4	0.119	44.2	85.0	74.5	0.154	6.2
21	CDC Dawn	34.3	0.134	43.9	94.1	78.5	0.151	6.7
22	CDC Sisler	39.4	0.109	61.6	95.1	89.2	0.138	8.5
23	Turk	25.1	0.107	34.7	74.3	59.1	0.125	5.2
24	Phoenix	26.2	0.122	35.1	80.1	71.6	0.116	5.3
25	Manley	36.5	0.102	55.5	94.5	90.5	0.132	7.3
26	CDC Stratus	33.0	0.129	46.9	94.6	77.6	0.163	6.7
27	Tercel	36.6	0.129	50.2	90.3	76.4	0.150	6.5
28	Duel	33.1	0.140	48.3	94.9	86.9	0.140	7.3
29	AC Albright	36.4	0.121	51.1	90.6	78.0	0.146	6.3
30	Bronco	14.2	0.027	22.5	58.1	49.6	0.030	5.4
31	B1602	41.5	0.134	59.8	93.5	74.6	0.153	7.3
32	TR139	37.5	0.122	53.3	91.0	83.5	0.139	7.4
33	AC Lacombe	30.9	0.182	39.2	79.1	62.5	0.246	5.7
34	CDC Dolly	20.3	0.123	24.6	74.5	54.0	0.149	4.5
35	Harrington	34.6	0.154	45.3	90.8	70.1	0.177	6.7
36	Modoc	26.2	0.137	34.9	93.2	72.1	0.167	5.2
37	AC Harper	20.1	0.095	26.9	81.2	57.4	0.144	4.3
38	Trebi	21.7	0.144	24.1	68.7	51.6	0.137	4.7
39	Osiris	16.8	0.173	17.0	34.2	49.1	0.131	3.4
40	AC Hawkeye	24.6	0.111	31.3	83.1	62.6	0.163	4.7
41	Brier	29.4	0.133	38.4	93.3	68.7	0.175	5.5
42	Duke	31.4	0.128	45.8	94.9	76.4	0.161	6.8
43	CDC Gainer	33.2	0.108	45.6	94.7	85.7	0.151	7.2
44	Zavila	14.3	0.093	16.6	54.0	37.4	0.157	2.7
45	AC Rosser	33.7	0.118	49.9	92.9	81.1	0.151	6.8
46	Stander	35.7	0.125	48.2	88.4	76.4	0.155	6.4
47	CDC Fleet	36.5	0.157	47.5	93.6	70.0	0.180	6.9
48	Foster	34.5	0.142	48.5	90.7	68.8	0.171	7.1
49	Brier (D)	27.8	0.119	38.5	88.9	72.9	0.165	5.7
50	Kasota	17.9	0.118	19.9	51.8	55.9	0.214	3.9

† Ent.: Entry. Numbers 1-50 represents cvs. CDC Silky to Kasota at Lacombe, respectively.

‡ FS= Final %severity; AS= Standardized area under disease progress curve (SAUDPC) for %severity; rS= Apparent infection rate (r) for %severity; FI= Final %incidence; AI= SAUDPC for %incidence; rI = r for %incidence; and TS: Terminal Severity (0-9).

Table 3-5. Least square means of barley cultivars/lines for all traits tested at Toluca, 2000-2001.

		Trait‡						
Ent†	Cultivar/Line	FS	rS	AS	FI	AI	rI	TS
1	CDC Silky	37.7	0.225	44.4	65.7	60.4	0.180	5.2
2	Bridge	40.4	0.066	54.2	68.7	67.5	0.049	5.3
3	CDC Guardian	58.2	0.136	70.7	91.3	83.1	0.031	7.2
4	Leduc	22.3	0.074	22.5	44.9	42.4	0.106	3.4
5	CDC Earl	60.4	0.237	67.7	79.1	72.3	0.068	7.5
6	WW x G	29.1	0.104	28.1	58.5	48.2	0.165	3.9
7	Falcon	30.4	0.106	30.2	52.4	45.3	0.138	5.1
8	Stein	61.6	0.130	77.9	93.1	86.5	0.023	7.7
9	AC Oxbow	63.2	0.139	76.0	92.2	84.0	0.040	7.8
10	AC Bountiful	52.0	0.123	63.4	83.3	76.1	0.040	6.5
11	B1215	58.5	0.102	73.4	92.9	89.2	0.025	7.7
12	UNA80	12.7	0.317	5.7	26.6	9.8	0.352	2.3
13	AC Metcalfe	53.7	0.109	66.1	87.3	76.5	0.028	6.7
14	TR145	49.7	0.096	61.4	86.2	77.2	0.045	6.6
15	Atlas46	57.2	0.089	72.7	91.0	93.3	0.021	7.1
16	Stetson	27.6	0.105	28.6	58.8	50.8	0.184	4.1
17	La Mesita	11.2	0.216	4.1	14.0	3.5	0.214	2.2
18	Jackson	60.5	0.063	79.8	93.0	92.9	0.010	7.5
19	Atlas	61.6	0.109	77.7	94.2	94.5	0.030	7.7
20	Tukwa	48.9	0.105	59.3	83.0	77.0	0.033	6.6
21	CDC Dawn	67.2	0.113	85.8	96.5	92.1	0.017	8.2
22	CDC Sisler	65.0	0.122	84.0	92.9	91.7	0.016	7.9
23	Turk	38.7	0.096	43.9	73.2	70.0	0.065	5.1
24	Phoenix	39.9	0.081	45.0	75.1	65.5	0.142	5.2
25	Manley	59.9	0.160	70.3	93.8	84.5	0.031	7.3
26	CDC Stratus	51.2	0.084	65.6	87.3	80.4	0.028	6.8
27	Tercel	56.6	0.112	67.1	89.9	80.5	0.029	7.1
28	Duel	49.4	0.052	66.3	84.5	80.9	0.015	6.4
29	AC Albright	44.1	0.028	58.5	75.5	73.7	0.096	5.6
30	Bronco	20.7	0.051	21.2	41.0	34.0	0.042	3.8
31	B1602	52.1	0.077	64.3	89.1	88.0	0.021	6.5
32	TR139	64.4	0.131	77.9	91.4	85.4	0.033	7.6
33	AC Lacombe	33.9	0.086	38.8	64.6	58.7	0.148	4.8
34	CDC Dolly	63.6	0.113	81.1	95.0	90.6	0.025	8.0
35	Harrington	58.9	0.094	77.4	91.2	89.8	0.026	7.5
36	Modoc	15.2	0.134	13.5	25.8	21.5	0.137	2.4
37	AC Harper	1.0	0.063	0.4	2.0	0.7	0.063	0.5
38	Trebi	16.9	0.228	7.7	23.3	11.6	0.241	2.9
39	Osiris	0.7	0.000	0.0	2.2	0.0	0.001	0.1
40	AC Hawkeye	4.8	0.066	4.4	10.3	5.5	0.058	1.5
41	Brier	30.3	0.071	16.9	57.4	19.9	0.098	4.1
42	Duke	53.7	0.108	68.3	88.6	79.8	0.050	7.3
43	CDC Gainer	59.0	0.124	72.5	87.4	81.3	0.028	7.3
44	Zavila	20.6	0.083	19.5	41.3	31.1	0.095	2.8
45	AC Rosser	39.3	0.091	45.3	75.4	66.8	0.125	5.5
46	Stander	41.8	0.046	57.6	73.8	71.0	0.017	5.5
47	CDC Fleet	65.8	0.138	77.9	95.1	91.8	0.012	8.1
48	Foster	53.3	0.076	71.4	87.8	86.4	0.020	7.1
49	Brier (D)	30.7	0.047	36.2	59.3	51.8	0.057	4.0
50	Shyri	3.4	0.158	19.5	10.4	35.8	0.203	1.4

† Ent.: Entry. Numbers 1-50 represents cvs. CDC Silky to Shyri at Toluca, respectively.

‡ FS= Final %severity; AS= Standardized area under disease progress curve (SAUDPC) for %severity; rS= Apparent infection rate (r) for %severity; FI= Final %incidence; AI= SAUDPC for %incidence; rI = r for %incidence; and TS: Terminal Severity (0-9).

Table 3-6. Factor analysis of the studied traits for the western Canadian barley cultivars in all locations/years. (A) Total variance explained using all extracted principal components (PCs). (B) Component matrix extracted for the PCs exhibiting Eigen value ≥ 1.

A

		Sums of squared loadings	
Component	**Eigen value**	**Variance (%)**	**Cumulative (%)**
1	4.88	69.76	69.76
2	1.67	23.89	93.64
3	0.25	3.58	97.22
4	0.13	1.91	99.14
5	0.04	0.52	99.66
6	0.02	0.27	99.93
7	0.01	0.07	100.00

B

	Component	
Trait	**1**	**2**
Final %severity (%S)	0.985	-0.094
Apparent infection rate for %S	0.396	0.857
Standardized area under disease progress curve for %S	0.967	-0.12
Final %incidence (%I)	0.951	0.02
Standardized area under disease progress curve for %I	0.967	-0.063
Apparent infection rate for %I	-0.078	0.954
Terminal severity	0.987	-0.015

Table 3-7. Pearson coefficient analysis of the studied traits and the extracted two principal components (PCs).

Trait	rS	AS	FI	AI	rI	TS	PC1	PC2
FS = Final %severity (%S)	0.332*	0.963**	0.927**	0.929**	-0.19	0.980**	0.985**	-0.09
rS = Apparent infection rate for %S		0.296*	0.334*	0.295*	0.692**	0.381**	0.396**	0.857**
AS = Standardized area under disease progress curve for %S			0.861**	0.963**	-0.20	0.937**	0.967**	-0.12
FI = Final %incidence (%I)				0.907**	-0.01	0.949**	0.951**	0.02
AI = Standardized area under disease progress curve for %I					-0.10	0.933**	0.967**	-0.06
rI = Apparent infection rate for %I						-0.10	-0.08	0.954**
TS = Terminal severity							0.987**	-0.02
PC1								0.00

* and ** Significant at the 0.05 and 0.01, respectively.

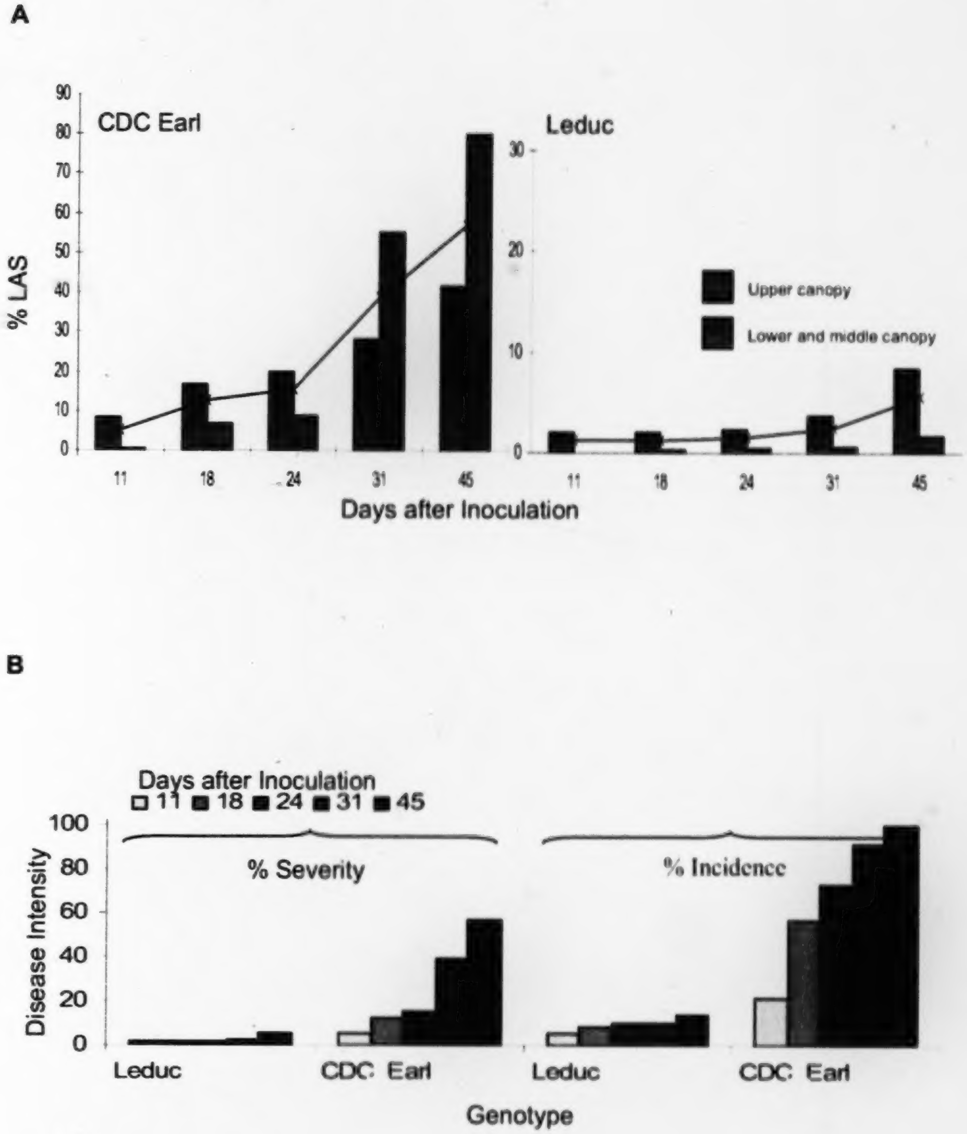

Figure 3-1. Disease progress based on (A) the percentage leaf area scalded (%LAS) in different canopy levels where the lines indicate average %LAS for all canopy levels, and (B) the disease intensity for the cultivars, CDC Earl (susceptible) and Leduc (slow-scalder) in Edmonton, 2001.

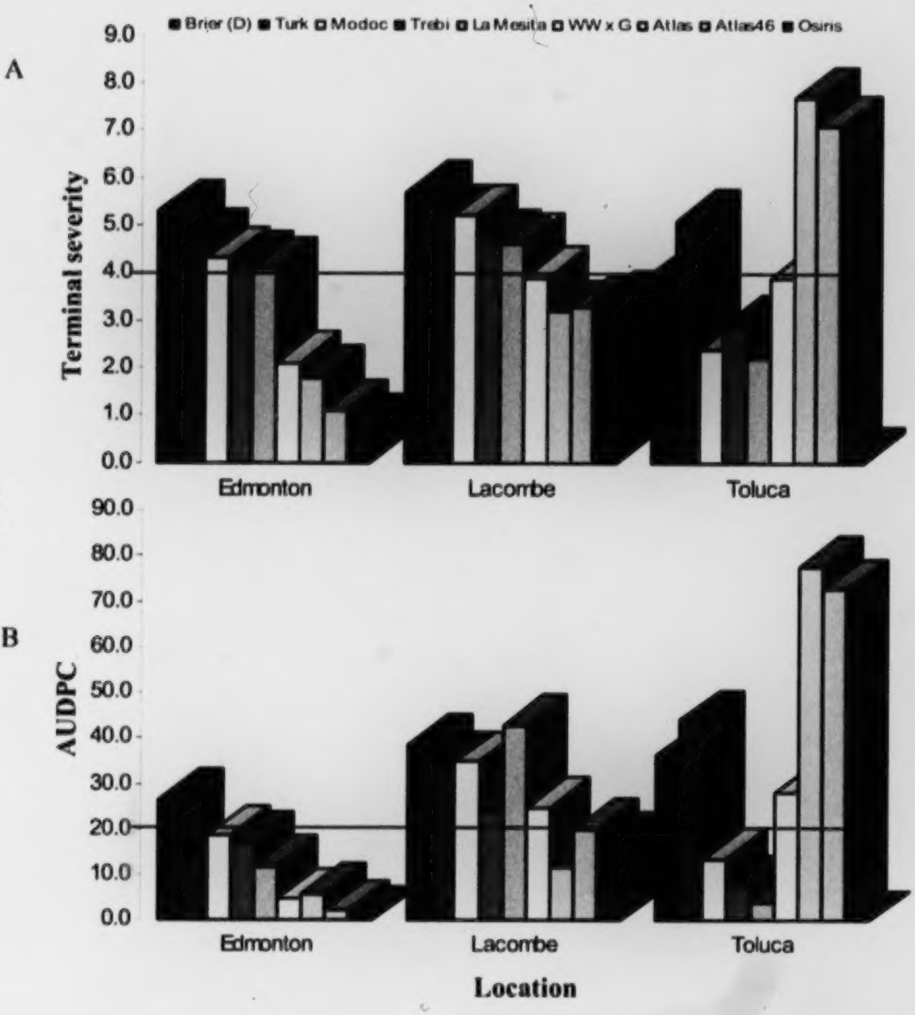

Figure 3-2. Disease reactions of barley scald differential genotypes based on (A) the disease score (0-9) and (B) the amount of scald progress on them (area under the disease progress curve, AUDPC) in Edmonton, Lacombe, and Toluca.

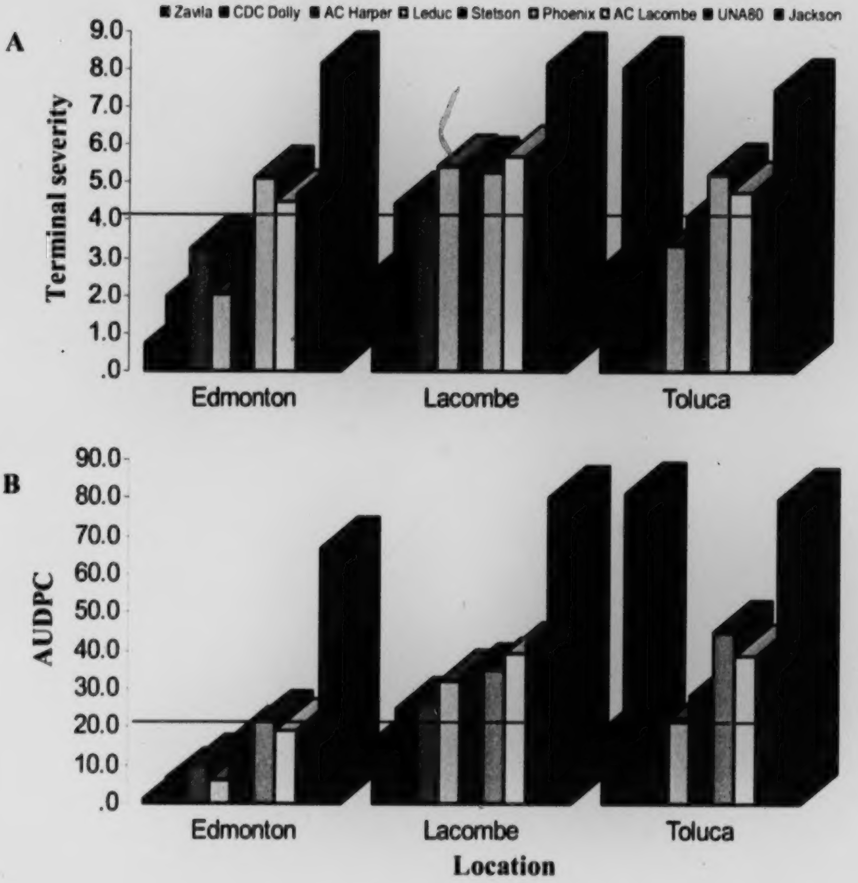

Figure 3-3. Disease reactions of some barley genotypes based on (A) the disease score (0-9) and (B) the amount of scald progress on them (area under the disease progress curve, AUDPC) in Edmonton, Lacombe, and Toluca.

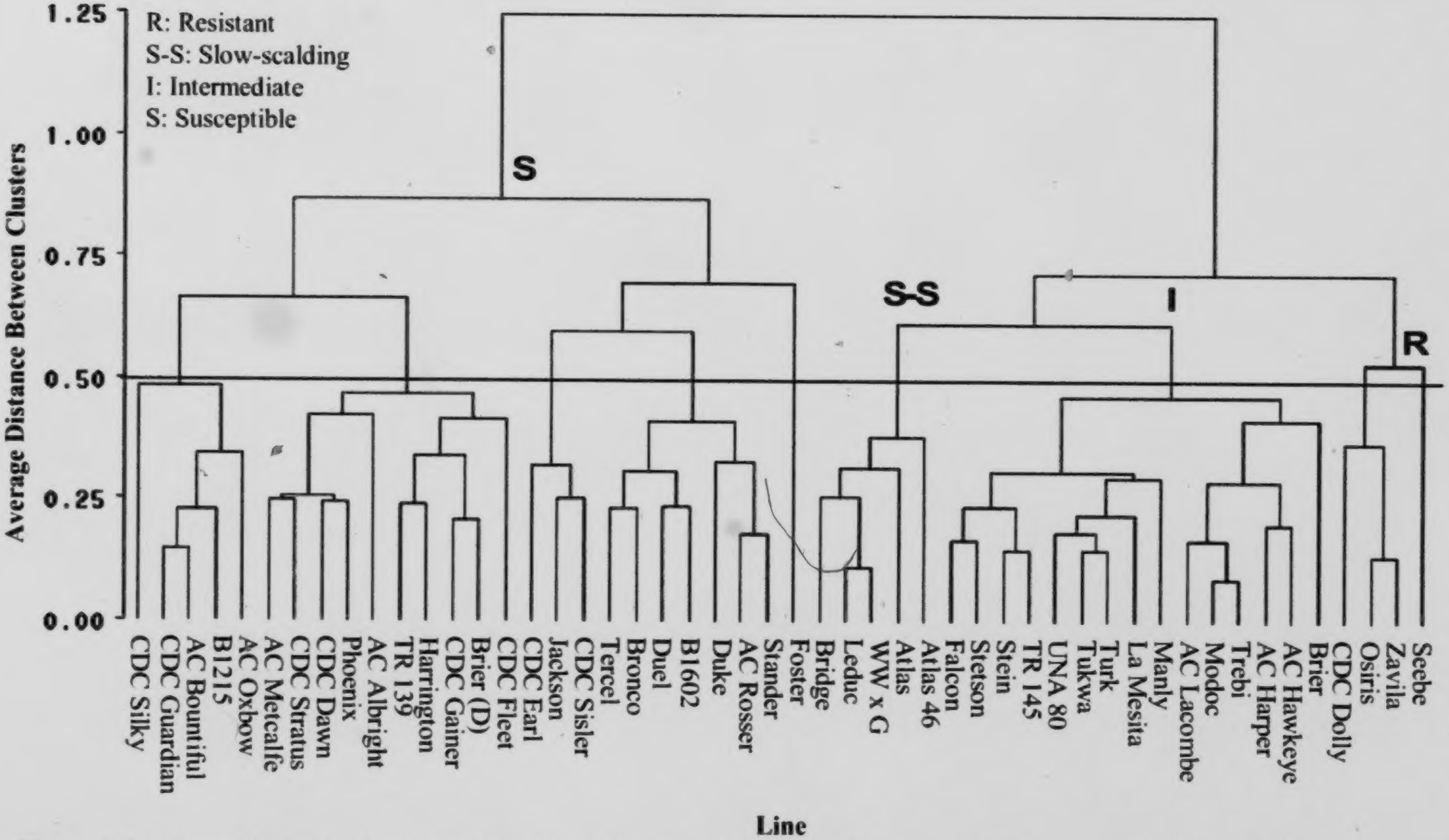

Figure 3-5. Hierarchical classification of 50 cultivars/lines based on their final percentage of severity (%S), standardized area under disease progress curve (SAUDPC) for %S, final percentage incidence (%I), SAUDPC for %I, apparent infection rate (r) for %S and terminal severity (TS) in Edmonton, where Seebe was included as a local resistant check. The red line shows arbitrary separation points.

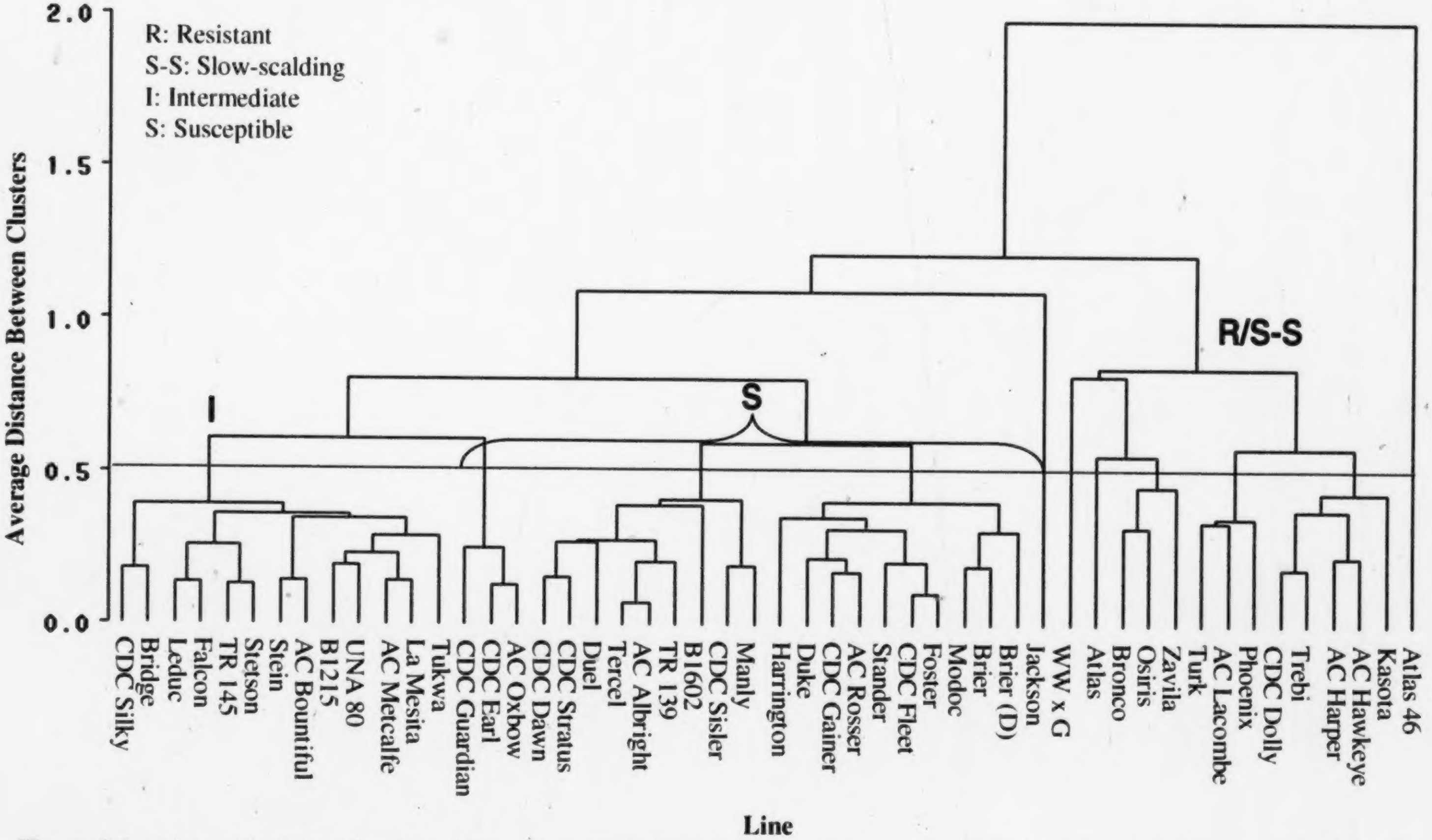

Figure 3-5. Hierarchical classification of 50 cultivars/lines based on their final percentage of severity (%S), standardized area under disease progress curve (SAUDPC) for %S, final percentage incidence (%I), SAUDPC for %I, apparent infection rate (r) for %S and terminal severity (TS) in Lacombe, where Kasota was included as a local resistant check. The red line shows arbitrary separation points.

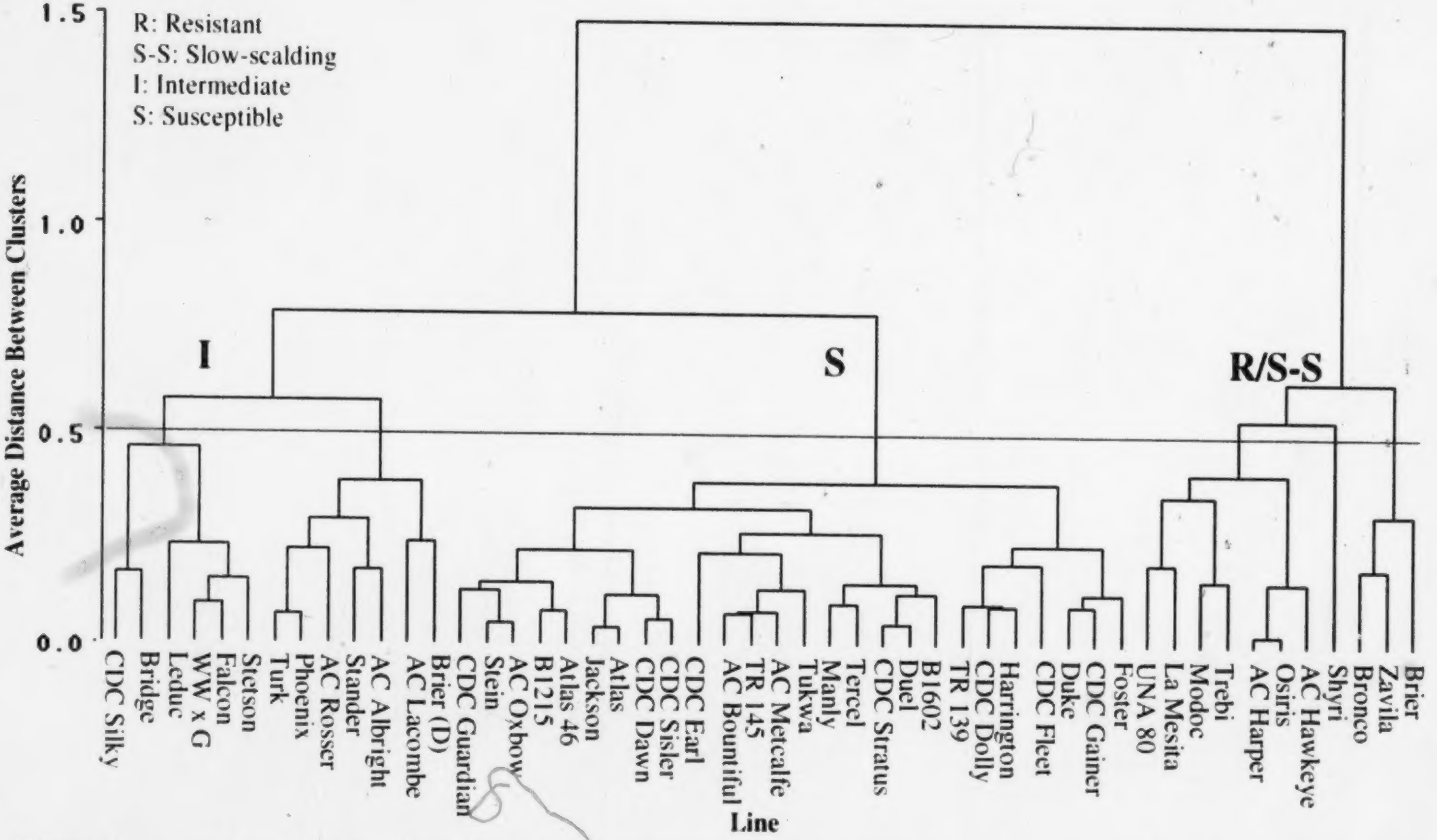

Figure 3-6. Hierarchical classification of 50 cultivars/lines based on their final percentage of severity (%S), standardized area under disease progress curve (SAUDPC) for %S, final percentage incidence (%I), SAUDPC for %I, apparent infection rate (r) for %S and terminal severity (TS) in Toluca, where Shyri was included as a local resistant check. The red line shows arbitrary separation points.

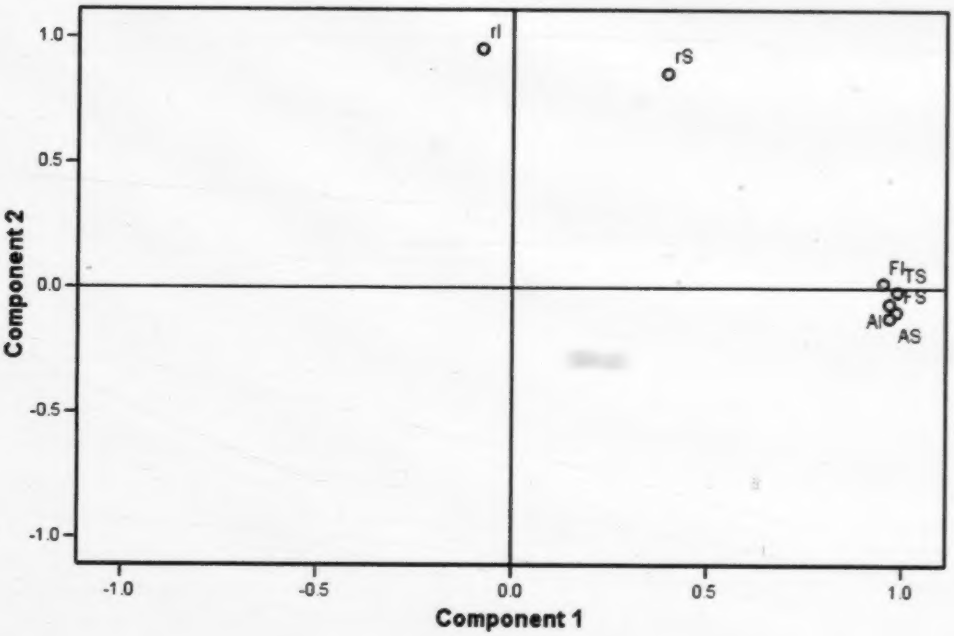

Figure 3-7. Component plot for FS: the final percentage of severity (%S), AS: standardized area under disease progress curve (SAUDPC) for %S, FI: final percentage incidence (%I), AI: SAUDPC for %I, rS: apparent infection rate for %S, rI: apparent infection rate for %S, and TS: terminal severity (0-9) based on two principal components accounting for 93.6% of the total variation among genotypes tested in all locations/years.

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Chapter 4

Pathogenicity of scald isolates on spring barley cultivars[§]

4.1. Introduction

Barley scald caused by *Rhynchosporium secalis* (Oud.) J. J. Davis, is an important foliar diseases of barley world-wide (Mathre, 1997). In Alberta, where approximately 5.68 out of a total 12.03 million tons of barley production in Canada is accounted for (Anonymous, 2003), the pathogen has been reported to be highly variable (Xi *et al.*, 2002) and causes considerable yield losses on the susceptible cultivars (Anonymous, 1999; Turkington *et al.*, 1998). In several cases the resistance in barley cultivars has been overcome a few years after registration (Xi *et al.*, 2003; Xue *et al.*, 1991). Even in newly developed pure lines, resistance has been reported to be broken by pathogen before they reach large areas of production (Yahyaoui, 2002). This is mainly due to favorable conditions for the development of scald, the highly pathological variable nature of *R. secalis* and continual changes in its populations, and high selection pressures resulting from resistant varieties which are cultivated consecutively in a particularly area (Tekauz, 1991). Hence, updated knowledge about pathogenic variation and pathogenicity of existing *R. secalis* isolates in a given area seems critical for disease management. Knowledge from studies on the reactions of barley genotypes to local sets of scald pathotypes may assist barley breeders and pathologists in resistance gene deployment strategies. Also, knowledge of interactions between barley genotypes and a wide set of *R. secalis* isolates is an essential aspect of breeding for durable resistance.

Although there is no agreement as to how to address questions about possible sources of variation among the pathogen isolates (Goodwin *et al.*, 1994; Konovalova, 2001a; b; Newman and Owen, 1985; Newton, 1989; Salamati *et al.*, 2000), there are many studies that demonstrate extreme pathogenic variability of *R. secalis* in different parts of the world (Goodwin *et al.*, 1993; Jackson and Webster, 1976; Jörgensen and Smedegaard-Petersen, 1995; McDermott *et al.*, 1989; McDonald *et al.*, 1999; Tekauz, 1991; Xue *et al.*, 1991). The first report on physiologic specialization in *R. secalis* was about 6 specialized races of the pathogen, which could be distinguished on different grass species differentials (Caldwell, 1937). Perhaps the first attempt to determine variation among scald isolates and to recognize scald races on barley differentials goes back to 58 years ago (Sarasola and Campi, 1947). The presence of races in the United States and Canada was reported by Reed (1957). Since then, numerous studies have been undertaken to elucidate differences among scald isolates based on their virulence and pathogenicity on sets of various barley genotypes (Brown, 1990; Robbertse *et al.*, 2000; Salamati and Tronsmo, 1997; Schein, 1958; Williams and Owen, 1973). Brown (1990) studied the pathogenicity of 276 single-spore isolates of *R. secalis* on 15 barley varieties having different combinations of resistance genes and showed a larger contribution of scald isolates from *Hordeum leporinum* than from those of *H. vulgare* in the observed pathogenic variation. They also found that there was as much pathogenic variation among single-spore isolates from the same lesion as among isolates from different lesions from samples collected from the same or different locations. This was in contrast with the conclusion drawn form another pathogenic variation study on isolates of scald where, using an AFLP marker system, close genetic similarity was found between isolates collected from the same scald "hotspot" in comparison to a higher level of variation between isolates from different "hotspots" (Williams *et al.*, 2003). In Canada, the first report indicated no pathogenic variability among 22 western Canadian isolates of scald collected from the prairie provinces (Skoropad, 1960). However, Xue *et al.* (1991) noticed pathogenic variation among isolates of *R. secalis* from Ontario. Tekauz (1991) studied reactions of 20 host cultivars including 10 differentials, which contained known scald resistance genes, to 111 Canadian isolates of the pathogen and identified 45 different pathotypes among the isolates studied. The results from this study confirmed great variability in *R. secalis* in Canada.

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Using standard differential barley genotypes with known resistance genes, coupled with their respective scald pathotypes/races having matching virulences, plant breeders and pathologist can study pathogenic variability of *R. secalis* on a local, regional, and global basis. It could also be a sound way to carry out genetic analysis and to differentiate among various barley genotypes or the pathogen isolates (Bjornstad *et al.*, 2000; Dyck and Schaller, 1961; Webster *et al.*, 1980). The expression of a given resistance gene may be strongly influenced by the pathogenic variability of *R. secalis* and by the environmental conditions under which the experiments are carried out, resulting in differences in reaction of that gene under various circumstances. Bjornstad *et al.* (2000) pointed out a number of possible factors causing discrepancies between the results of different studies on the barley-scald pathosystem. They indicate heterogeneity in a given differential line's seed source, differences in the pathogenicity of isolates used, variable effects of environmental conditions on expression of a given gene, and misinterpretation of some findings. They concluded that "…a revision of previously published data on the genetics and designation of resistance to scald is warranted". To achieve this, near-isogenic lines (NILs) for resistance to barley scald were developed using a susceptible cultivar Ingrid as the recurrent parent and a set of barley differentials as the resistant donor parents (Bjornstad *et al.*, 2002). The studies on marker and phenotypic analyses of NILs have resulted in an updated terminology of genes for resistance to *R. secalis* (Patil *et al.*, 2002), which grouped genes conditioning resistance in the differentials Turk, Brier, La Mesita, Atlas 46, Modoc, Hudson, and Abyssinian as alleles of a complex locus, Rrs1. However, these results were not in total agreement with the information obtained from previous findings, nor were they deemed satisfactory to be adopted as a new nomenclature of the scald resistance genes (Wallwork, 2002).

In general, there has been much conflicting information about the resistance genes for scald which might primarily be due to different responses of barley differentials under different environmental conditions and various arrays of *R. secalis* isolates with different virulence patterns. Therefore, it is important to consider the effects of genotype by environment interaction (G x E) in causing such variation and its implication on durable resistance breeding (Pinnschmidt and Rasmussen, 2002a). The G x E interaction can be explained by the direct effects of environmental conditions on either the plant or the pathogen, or the interactions between specific resistance traits in the test plant materials and corresponding virulence/avirulence features in the local scald populations

(Pinnschmidt and Rasmussen, 2002c). The potential impact of G x E can be reduced by conducting barley-scald experiments under controlled environments (Xi *et al.*, 2003), although, indoor experiments are not always feasible. Lasting resistance is difficult to achieve against pathogen populations with diverse arrays of virulence genes (McDonald *et al.*, 1988). However, exposing a given cultivar to a wide spectrum of pathotypes/races of the scald pathogen under optimal conditions conducive for development of the disease could provide barley workers with information pertaining to stability/durability of resistance. In an international effort (Scald Ring Test), barley-scald workers in Europe, New Zealand, and Australia have collaborated to assess scald resistance of their spring barley materials over a total of 32 environments in 8 countries with 16 locations during the summers of 1999-2001 (Pinnschmidt and Rasmussen, 2002b). The results showed that the resistance performance in most cultivars were unstable and have been affected by G x E interactions. The lack of matching virulences in populations of *R. secalis* to resistance genes in Atlas 46, Abyssinian, and CI 4364 was considered to be the reason for observed low levels of disease on these differentials under all circumstances.

Isolates of a disease causing agent are routinely characterized by their spectrum of pathogenicity (virulence and aggressiveness) for a defined set of differential host lines (Herrmann *et al.*, 1999), and are named as different pathotypes/races using various nomenclatures (Gilmour, 1973; Goodwin *et al.*, 1990; Habgood, 1970). In pathogenicity tests, “virulence” and “aggressiveness” are the main components of pathogen fitness (Welz, 1988), where the former is defined as a qualitative trait related to race-specific resistance genes and the latter term represents the quantitative ability of a pathogen to cause disease (Van der Plank, 1975). Different studies have been undertaken to examine virulence and aggressiveness of *R. secalis* isolates originating from different parts of the world. However, neither the pathogenic variation in isolates from the “scald hot spot”, Toluca, Mexico, nor the reactions of a set of different differentials, and Canadian and ICARDA/CIMMYT cultivars/lines to a set of Canadian and Mexican isolates have so far been tested. The objectives of the present study were to examine the pathogenicity of a set of such *R. secalis* isolates, and to determine the potential durability of such barley material. The information derived would be of value for planning durable resistance breeding.

4.2. Materials and Methods

4.2.1. Plant materials

A set of 9 differentials, 44 western Canadian, and 20 ICARDA/CIMMYT barley cultivars/lines were selected to be examined against 19 isolates of the fungus (Table 4-1). The 44 Canadian barley cultivars/lines were a sizable representative of genotypes with susceptible, intermediate, and resistant reactions to scald in western Canada. The 20 ICARDA/CIMMYT barley genotypes were all selected as potential sources of resistance to scald from different CIMMYT barley breeding programs except for the cultivar, Cerise which was a highly susceptible cultivar. The 9 out of 19 such resistant ICARDA/CIMMYT genotypes have been reported to carry a slow-scalding type of resistance (Ginkel and Vivar, 1986). In this study, the proposed core set of barley differential lines (Tekauz, 1995) was also included, except for the winter barley cv. Hudson.

4.2.2. Scald single-spore isolates

Isolation and culture of *R. secalis* was performed according to procedures outlined in Chapter 1. The fungal materials collected in this research were more than 250 single-spore isolates of *R. secalis* from different locations/years in Alberta, Canada and one location in Toluca, Mexico. From this, only a set of 6 Canadian and 13 Mexican isolates of the pathogen were used in the present study (Table 4-2).

4.2.3. Seedling tests

The plant materials were separately spray-inoculated with the prepared inoculum of each isolate and a routine seedling test (Chapter 2) with 3 replicates was performed to evaluate reactions of all 73 barley genotypes to a set of 19 scald isolates as outlined in Chapter 2. The reactions of barley materials to scald isolates were recorded using the 0-4 and 0-9 scales proposed in Chapter 2.

4.2.4. Statistical procedures

The experiment was conducted twice and average values were used for statistical analyses. The obtained resistance/virulence data were used to determine resistance and pathogenicity of the plant and fungal materials, respectively, where a score of 2.5 or greater was an indication of susceptible reaction. The Manhattan Metric (MM) was calculated as a criterion of dissimilarity/distance between isolates (Sneath and Sokal, 1973):

$$M = \sum_{K=1}^{s} \left| X_{ik} - X_{jk} \right|$$

where X_{ik} and X_{jk} are the average disease reactions of *K*th cultivar/line challenged with *i*th and *j*th isolates, and *s* denotes the total number of cultivars that is 73. The calculated dissimilarity measures were further used for the classification of studied isolates. The hierarchical cluster analysis was performed using the CLUSTER procedure by the average linkage method (unweighted pair-group method using arithmetic averages, UPGMA) and the resulting dendrogram was illustrated using the Tree procedure (SAS Institute Inc., 1989). Another classification of the 19 isolates was performed by cluster analysis using the average reactions of all the 73 barley genotypes to each scald isolate.

Disease index was calculated as explained by Salamati and Tronsmo (1997) as follows:

$$DI = \sum_{i=0}^{4} i f_i \Big/ \sum_{i=0}^{4} f_i$$

where DI is the mean of scores weighted by the highest score (i.e. 4), and f_i is the number of scores in the *i*th category. DI was calculated both for all isolates and genotypes. The values of DI for the isolates were used. Likewise, aggressiveness of different isolates were calculated using means of all ratings greater than 2.5 which were then weighted by the number of scores for each isolate. Analyses of variance were undertaken for the aggressiveness and disease indices, and Duncan's multiple range tests were used to differentiate among significantly different isolates. A cluster analysis was applied to the DI calculated for the genotypes to classify them based on their overall performance to all isolates evaluated.

4.3. Results

Table 4-1 shows a set of 9 differentials, 44 Canadian and 20 ICARDA/CIMMYT barley cultivars/lines used in this study. The single-spore isolates used in this research were named based on their Dr. J. P. Tewari's University of Alberta's collection identities (Table 4-2), and characterized based on their virulence and aggressiveness on a set of 73 barley genotypes. All isolates tested were collected from experimental plots in Canada and Mexico. Reactions of barley scald differentials to these isolates of *R. secalis* are shown in Table 4-3. None of the barley differentials reacted similarly to the pathogen isolates. As shown in Table 4-3, barley differentials differed in the number of isolates to which they were susceptible.

Osiris and La Mesita were only susceptible in 3 and 4 cases, respectively, whereas the rest of the differentials showed susceptible reactions to at least 10 out of 19 isolates studied. None of differential genotypes was susceptible to Iso 1 whereas all of them were susceptible to Iso 7. The isolates Iso 2 and Iso 16 caused susceptible reactions only on Trebi and Atlas, respectively whereas Iso 10 and Iso 19 were virulent on all differentials except against La Mesita. Among the Canadian isolates, Iso 1 had no virulence factor affecting the differentials to cause susceptibility. Iso 2 seemed to carry a matching virulent gene breaking resistance conditioned by a resistance gene in Trebi which was not susceptible to other Canadian isolates. Iso 5 had a complex array of virulence and was shown to carry the same factors as causing disease in Iso 3 and Iso 6. However, it caused different reactions on Atlas, Atlas 46, and La Mesita as compared to those of Iso 4. To identify these isolates as different pathotypes/races and also for the sake of uniformity in future researches, the isolates were named using the nomenclature of Goodwin *et al.* (1990) for *R. secalis*. As shown in Table 4-3, the isolates with different octal names exhibited differential reactions for the scald barley differentials and were identified as 15 different pathotypes.

Although, based on the information from the reactions of differentials (Table 4-3), some pathotypes had the same pathogenicity, and the data obtained from other 64 barley cultivar/lines studied confirmed that they were all different (Table 4-4). Different superscripts, ^{a} or ^{b}, were used to differentiate between the pathotypes with the same octal name. Table 4-4 provides a summary of the characteristics of all identified pathotypes. For each category of barley genotypes and for each pathotypes, the number of susceptible reactions observed were presented. For a given pathotype, the total numbers of susceptible interactions out of 73 genotypes studied were calculated as the percentage barley genotypes susceptible to the *R. secalis* isolates studied (%S). On the other hand, to show pathogenicity of the pathotypes, disease index (DI) and aggressiveness (Agg) of each of them were also included in Table 4-4. For the sake of simplicity, instead of the octal name, isolate number is used hereafter. For example, Iso 19 is used to describe the pathotypes "_____5735^{a}". The results showed that the pathotypes had different virulence/aggressiveness on the barley genotypes studied. Interestingly, isolates collected from the same host within an experiment (Iso 7 and Iso 8; Iso 11, Iso 12, and Iso 13; Iso 18 and Iso19) were significantly different for their aggressiveness and disease index. Based on the results obtained, about 92% of the 73 barley genotypes studied were susceptible to Iso 19 (collected from the cultivar Seebe in Mexico). In contrast, only 8 genotypes exhibited differential responses to Iso 1. These pathotypes were identified as the most and least aggressive isolates of scald studied in this experiment, respectively. The rest of the pathotypes were virulent on 25 to 65 out of 73 genotypes challenged with. Some pathotypes characterized by the same %S, DI, or Agg, however, neither made the same number of susceptible reactions in each barley category, nor showed similar pathogenicity patterns as revealed by both DI and Agg.

As shown in Table 4-5, the genotypes were characterized using their reactions to the isolates studied. In this table, a summary of reactions to all 19 isolates, the number of susceptible reactions (No.S), and the disease indices calculated for all genotypes are shown. The results showed that none of the barley genotypes were immune against all the isolates. Among the differentials, Osiris and La Mesita showed the lowest disease indices whereas Brier and Atlas and WW x G showed susceptibility to the largest number of the isolates studied and had the highest DI. The most resistant genotype, Laurel was an

ICARDA/CIMMYT genotype. Next to Laurel (BYDV resistant), were AC Hawkeye (hulless), Calicuchima.92 (malting), API/CM67-B//AGER (slow-scalding), CSBN 8527 (leaf rust resistant), and Kasota and AC Harper (general purpose). In general, the majority of genotypes with the lowest DI were form the ICARDA/CIMMYT barley breeding program. In contrast, Jackson, Tercel, and Harrington were susceptible to all isolates and showed the highest level of susceptibility as determined by DI. Cerise, as a ICARDA/CIMMYT susceptible check, was only resistant to Iso 16 which indeed was the least virulent isolate among the Mexican pathotypes studied. Among slow-scalding barleys, UNA 80 showed a disease index of 0.68 equal to that of CDC Dolly where they were only resistant to 6 and 5 out of 19 isolates studied. UNA 80 was resistant to more Mexican isolates whereas CDC Dolly showed lower levels of susceptibility to Canadian isolates. However, both genotypes had higher DI's in comparison with those of other slow-scalding genotypes investigated during the present study. UNA 80 showed the highest value for DI where API/CM67-B//AGER, Joso, were among the least susceptible genotypes. Among ICARDA/CIMMYT slow-scalding lines, Zavila showed an intermediate amount of DI and a number of susceptible reactions to the isolates tested where disease indices of CI1240/Foma//CI16239.15D, and ORGE618 had the closest DI's to that of UNA 80. The resistance patterns in these genotypes were quite different. K8755 and Zavila showed more similarities and were only different for Iso 1, Iso 2, Iso 8, and Iso 9. Among the WCBC with the lowest DIs, AC Hawkeye and Kasota, AC Harper, CDC Richard, and Mahigan (and Seebe) showed close relationships with the resistance patterns of Osiris, La Mesita, Zavila, and ORGE618, respectively. DI values in cvs. Duke, CDC McGwire, AC Metcalfe, Stetson, AC Lacombe, CDC Silky, and AC Oxbow were similar ranging from 0.56 for Duke to 0.66 for AC Oxbow.

In the present study, using cluster analyses different groups of isolate-pathogenicity (Figure 4-1) as well as of host resistance (Table 4-6) were recognized. Using MM, the isolates were grouped into two distinct clusters (Figure 4-1A). The Cluster I contained all Canadian isolates except the isolate 5. The most aggressive Canadian isolate, Iso.5 and all 13 Mexican isolates of *R. secalis* represented Cluster II. As shown in Figure 4-1B, cluster analysis also enabled us to differentiate among the isolates tested based on their average reactions on all the 73 isolates. To study isolates, a separation point was arbitrarily chosen forming 4 major clusters. The Iso1 alone made a one-entry cluster. However, with respect to its low amount of pathogenicity and also close relationship with other Canadian isolates, it was regrouped with another cluster into Cluster I. It is worth mentioning that among all Mexican isolates tested, Iso16 showed the least complexity and aggressiveness, and was classified with Canadian Iso.1, Iso.2, Iso.3, Iso.4, and Iso.6. Moreover, among all the Canadian isolates, Iso 5 was the most aggressive and grouped with Mexican pathotypes into Cluster III. Isolates with low amounts of pathogenicity were classified in clusters I and II, whereas the clusters III and IV consisted of isolates with higher levels of pathogenicity on most cultivars. The isolates in Cluster IV were characterized bys high pathogenicity, which caused susceptible reactions on most studied host genotypes. Clusters I, II, III, and IV showed a disease mean ± standard deviation of 1.6±0.45, 2.4±0.12, 2.8±0.08, and 3.3±0.07, respectively.

Hierarchical cluster analysis which was performed on the calculated disease index for barleys using the average linkage method resulted in classification of the plant materials into different resistance groups (Table 4-6). The cluster of "highly resistant" genotypes contained barleys resistant to the majority of pathotypes among which, there were some malting (Calicuchima 92), hulless (AC Hawkeye), slow-scalding (Joso), and differential (Osiris) lines. Lines with resistance to other diseases such as BYDV (Laurel) and leaf rust (Matico) were also found among this cluster. Based on their overall reactions to the all pathotypes, the differential genotypes, Brier, Turk and WW x G, Atlas 46 and Trebi, Modoc, and Osiris and La Mesita were found in clusters representing susceptible, moderately susceptible, moderately resistant, resistant, and highly resistant genotypes, respectively. The slow-scalding lines were found among the clusters, "Highly resistance" to "Moderately resistant". However, none of them was found among the clusters of moderately to highly susceptible genotypes.

4.4. Discussion

4.4.1 Classification of the scald isolates

There is a controversy among barley-scald workers whether to use the term "race" or not as differences in pathogenicity of different isolates may not be quite distinct and readily identifiable in all instances (Ali and Boyd, 1973). Some workers preferred to use the alternative terminology "pathotype" to avoid the problems associated with inter- and intra-isolate variability in *R. secalis* pathogenic characteristics (Dr. A. Tekauz, personal communication). In contrast, Ali and Boyd (1973) identified hosts expressing their resistance/susceptibility under different environmental conditions and isolates which could express their pathogenic characteristics consistently. In the present study, we used the term "pathotype" to name the isolates which were identified with different pathogenicity levels.

In this research, from a large number of leaf scald samples collected from different locations in western Canada and one location in Mexico, about 250 single-spore isolates of the pathogen were stored in the Dr. Tewari's University of Alberta scald single isolate collection. Since there were a number of studies on the pathogenic variation of *R. secalis* in Canada and almost no published data on the Mexican scald populations, we included 13 of the Mexican and only 6 of the Canadian isolates in this study. This study revealed that there were significant differences among the isolates studied in terms of their virulence and aggressiveness on different plant genotypes. As we saw, based on the reactions of nine differential lines and octal nomenclature of Goodwin *et al.* (1990), 15 pathotypes were initially identified. However, using disease index and aggressiveness calculated based on the reactions of all 73 barley genotypes, the isolates were differentiated into 19 different pathotypes. Identification of 19 different pathotypes out of 19 randomly selected isolates among a group of 250 single-spore isolates of the pathogen is indicative of a tremendous amount of pathogenic variability around the experimental plots where scald nurseries were conducted in both Canada and Mexico. Such information obtained could help plant pathologists to identify an array of different isolates which could represent pathogenic variability of the scald population at a given location. Consequently barley breeders would test their materials against this set of representative isolates and breed for more effective resistance against the pathogen. To breed for higher stability over different locations, a pathologist should expose the plant materials to different arrays of the pathogen representing the target locations, and a breeder should accumulate more resistance genes with higher stability over time. In order to deploy effective resistance gene(s) for each location, one should continuously monitor changes in the pathogen populations as well. A gene whose resistance has been broken, should not be used in areas where its corresponding virulent gene is prevalent, whereas if needs be, it can be deployed in regions where its respective race(s) does not occur. This study provides both plant breeders and pathologists with information about scald pathotypes from two important countries where breeding for durable scald resistance is a high priority.

In previous studies, different methods of cluster analysis and dissimilarity matrices were used as a useful means for grouping the pathogen isolates or their pathogenicity patterns on sets of various differentials (Brown, 1990; Xi *et al.*, 2002). In the present study, unweighted pair-group method (UPGMA) was used for classification of different pathotypes based on their MM dissimilarities and the average reactions of all genotypes studied. Fundamental differences were observed to exist between the Canadian isolates and those originating from Mexico, grouping them into distinct clusters representing different origins and pathogenicity levels. With respect to disease means of the 4 clusters identified (Cluster I, 1.55; Cluster II, 2.41; Cluster III, 2.77; Cluster IV, 3.28), a separation point of 2.5 was considered useful to differentiate among the pathogenicity of isolates. This was in accordance with the 0-4 seedling scale cut-off point and resulted in an appropriate separation of isolates based on their virulence pattern. From the data presented, it can be inferred that the western Canadian barleys likely carry resistance factors which are effective against Canadian isolates, whereas most Mexican isolates are highly virulent on them. Any kind of seed or plant parts import must be highly controlled to avoid a possible break down of effective resistances conditioned by Canadian sources of resistance.

4.4.2 Classification of barley genotypes

In the present study, none of the barley differentials showed the same resistance patterns to pathogen isolates. As mentioned earlier, differentials with the same reported genes were shown to have different reactions to the isolates tested. However, their reactions in some cases were similar and in accordance with the previous knowledge of their resistance gene(s). For example according to the information provided in Tables 1-1 and 1-2 of Chapter 1, the gene Rh2 in the short arm of chromosome 7H has been considered to condition resistance in Atlas and Atlas 46. In this study, these two differentials were shown to have the same reactions to 16 out of the 19 isolates and were different in 3 cases in which Atlas 46 was found to be more resistant. This was in accordance with previous reports (Dyck and Schaller, 1961; Starling *et al.* 1971) that Atlas 46 should have an additional locus for resistance, Rh3, at the chromosome 3H. Similar conclusions could be drawn for Atlas 46 and Turk which should carry Rh3, and must be different only for Rh2. Turk may also have Rh5 in addition to its Rh3 or rh6 (a possible recessive gene reported on the differentials, Osiris, Brier, and Modoc which has been designated on chromosome 4). Previous studies on these differentials indicated that Rh3 and Rh4 are closely linked on the complex locus Rrs1 or Rh/Rh3/Rh4 located at the short arm of the chromosome 3H (Dyck and Schaller, 1961 ;Williams *et al.*, 2001). This indicates that there might be similarities between the reactions of genotypes having Rh, Rh3, and Rh4 such as Turk, WW x G, and Brier. In this study, a close resistance pattern was found among these genotypes. Despite these similarities there were discrepancies between the differential reactions observed in these genotypes which in some cases could not lead to the same conclusions as those provided by some previous workers. For example, while Modoc, Trebi, La Mesita, and Osiris all are supposed to have Rh4 gene in common (Dyck and Schaller, 1961), their performance and resistance patterns were different where Osiris and La Mesita were observed to show only 3 and 4 cases of susceptibility, and Modoc and Trebi were found to be susceptible to 10 and 11 of the isolates, respectively. These findings support the idea that Osiris and La Mesita must have additional resistance factors as indicated by Habgood and Hayes (1971). The differentials Osiris and La Mesita have been considered to carry the Rh10 gene. Similarly, Brier with the Rh/Rh1 gene located at the long arm of the chromosome 3H, and WW x G carrying an allelic form of Rh, Rh^3, were also observed to react similarly in 16 cases, whereas they responded differently to 3 isolates. Considering the fact that there were no similarities between the isolates which differentiated between barley hosts with the same gene, it should be expected that there should be additional factors which cause differences in resistance levels among the genotypes with common resistant genes. Hypothetically, these differences could be due to additional resistance loci, separate effects of two closely linked genes, different genetic backgrounds, or the effects of modifying genes. Hence, studies on virulence in relation to specific host genes are more beneficial and informative if using a set of differentials in which each component possesses a single, unique resistance gene differing from others, and a uniform genetic background with no quantitative resistance to the disease (Welz, 1988).

It seems that the effectiveness of resistance factors in each genotype depends on the pathogenicity of local populations of scald in a given geographic area. It has been reported that Atlas 46 showed resistance to all Italian races while it was susceptible to some of the British scald isolates, whereas La Mesita was resistant to all British but not to Italian isolates (Ceoloni, 1980). Tekauz (1991) reported that Atlas 46 was resistant to the greatest number of 45 identified pathotypes. In the present study, Osiris and La Mesita showed the greatest number of resistant reactions whereas Atlas 46 was susceptible to 13 pathotypes. In another study, Jōrgensen and Smedegaard-Petersen (1995) identified 28 different races out of a total 38 Danish isolates of *R. secalis* tested among which no single races was virulent on Atlas and Osiris, and, Atlas 46 and La Mesita were susceptible to only two and three of the races.

It seems that a complex array of different alleles/genes must be involved in the pathogenicity of isolates studied here where most of the isolates could cause susceptibility on three or more differentials. To screen for horizontal resistance, application of a single race, which could differentiate for several resistance genes at a time, is highly recommended (Van der Plank, 1984). The importance of a single race with such a virulence pattern is two-fold if its corresponding resistance genes are representative of the geographical areas for which the barley cultivars are bred. With respect to the complexity of identified pathotypes e.g. Iso 19 and Iso 7 in Mexico, and Iso 5 in Canada, it looks beneficial to screen barley materials for durable resistance against such pathotypes of scald.

On the other hand, differences among the resistance patterns of those differentials with known genes and the plant genotypes evaluated in this research, could imply that there are even more genes to be identified and more resistance to be incorporated into the breeding lines of both Canadian and ICARDA/CIMMYT barley programs. The ICARDA/CIMMYT cultivars/lines used in his study have likely a core set of effective resistance genes from all different parts of the world as they are promising candidates in such international collaborative breeding levels, where extremely high pressures of disease epidemic are present. As rationalized for multilines or cultivar mixtures (McDonald *et al.*, 1988), barley differentials with two or more scald resistance genes (e.g. Osiris, Atlas 46, and Turk) could likely confer a more stable type of resistance against local spectra of the pathogen isolates. As a practical key breeding strategy, it is rationalized that genotypes with resistance against a higher number of isolates in the pathogen populations could perform in a more durable way. The results also indicated that genotypes with resistance to the majority of pathotypes were from all different categories e.g. malting (Calicuchima 92), hulless (AC Hawkeye), slow-scalding (Joso), and differential (Osiris). These highly resistant genotypes could be promising as potential sources of stable resistance to scald. Lines with resistance to other diseases such as BYDV (Laurel) and leaf rust (Matico) were also found among this cluster, which will be useful for multiple resistance breeding. In general, barley cultivar/lines in Clusters I, II , and III could be considered as useful germplasm for scald resistance.

4.4. Tables and Figures

Table 4-1. Barley cultivars/lines used in the pathogenicity test.

No.	Genotype / Parentage	Row type	Source*	Purpose used / Reaction to scald
1	Atlas	6	ACRS	Scald differential
2	Atlas46	6	ACRS	Scald differential
3	Brier	6	ACRS	Scald differential
4	La Mesita	6	ACRS	Scald differential
5	Modoc	6	ACRS	Scald differential
6	Osiris	6	ACRS	Scald differential
7	Trebi	6	ACRS	Scald differential
8	Turk	2	ACRS	Scald differential
9	Wisconsin Winter x Glabron	6	ACRS	Scald differential
10	Aleli	2	CIMMYT	Scald and BYDV resistant
11	Laurel	2	CIMMYT	Scald and BYDV resistant
12	Cardo	6	CIMMYT	Scald and BYDV resistant
13	Mejorana	6	CIMMYT	Scald and BYDV resistant
14	Madre selva	2	CIMMYT	Resistant
15	CSBN 8527	2	CIMMYT	Scald and leaf rust resistant
16	Gloria-Bar/Copal	6	CIMMYT	Scald and leaf rust resistant
17	Matico	6	CIMMYT	Scald and leaf rust resistant
18	Calicuchima.92	6	CIMMYT	Resistant
19	Zarza	6	CIMMYT	Resistant
20	K8755	2	CIMMYT	Slow-scalding
21	API/CM67-B//AGER	6	CIMMYT	Slow-scalding
22	CI1240/Foma//CI16239.15D	6	CIMMYT	Slow-scalding
23	Gatillo-Bar	6	CIMMYT	Slow-scalding
24	Joso	6	CIMMYT	Slow-scalding
25	Manker/ATHS-B	6	CIMMYT	Slow-scalding
26	ORGE618	6	CIMMYT	Slow-scalding
27	UNA80	6	CIMMYT	Slow-scalding
28	Zavila	6	CIMMYT	Slow-scalding
29	Cerise	2	CIMMYT	Susceptible
30	CDC Dawn	2	WCBC	Hulless / Susceptible
31	CDC Gainer	2	WCBC	Hulless / Susceptible
32	CDC McGwire	2	WCBC	Hulless / Intermediate
33	Phoenix	2	WCBC	Hulless / Susceptible
34	AC Hawkeye	6	WCBC	Hulless / Intermediate
35	CDC Silky	6	WCBC	Hulless / Intermediate
36	Falcon	6	WCBC	Hulless / Intermediate

*ACRS: Agriculture Canada Research Station, Winnipeg, Manitoba, Canada.
CIMMYT: International Maize and Wheat Improvement Center, Mexico.
WCBC: Western Canadian barley cultivars (Anonymous, 1989-2004).

Table 4-1. Continue …

No.	Genotype / Parentage	Row type	Source*	Purpose used / Reaction to scald
37	Bridge	2	WCBC	Susceptible
38	CDC Dolly	2	WCBC	Intermediate
39	CDC Fleet	2	WCBC	Susceptible
40	CDC Guardian	2	WCBC	Susceptible
41	Kasota	6	WCBC	Resistant
42	AC Albright	6	WCBC	Susceptible
43	AC Harper	6	WCBC	Intermediate
44	AC Lacombe	6	WCBC	Intermediate
45	AC Rosser	6	WCBC	Susceptible
46	Brier	6	WCBC	Intermediate
47	Bronco	6	WCBC	Susceptible
48	Jackson	6	WCBC	Susceptible
49	Leduc	6	WCBC	Intermediate
50	AC Bountiful (TR243)	2	WCBC	Malting / Susceptible
51	AC Metcalfe	2	WCBC	Malting / Susceptible
52	AC Oxbow	2	WCBC	Malting / Susceptible
53	B1215	2	WCBC	Malting / Susceptible
54	CDC Richard	2	WCBC	Hulless/ Resistant
55	CDC Stratus	2	WCBC	Malting / Susceptible
56	Harrington	2	WCBC	Malting / Susceptible
57	Manley	2	WCBC	Malting / Susceptible
58	Stein	2	WCBC	Malting / Susceptible
59	Tercel	2	WCBC	Malting / Susceptible
60	TR 251	2	WCBC	Malting / Susceptible
61	TR139	2	WCBC	Malting / Susceptible
62	TR145 (CDC Thompson)	2	WCBC	Malting / Intermediate
63	B1602	6	WCBC	Malting / Susceptible
64	CDC Sisler	6	WCBC	Malting / Susceptible
65	Duel	6	WCBC	Malting / Susceptible
66	Foster	6	WCBC	Malting / Susceptible
67	Stander	6	WCBC	Malting / Susceptible
68	CDC Earl	6	WCBC	Semi-dwarf / Susceptible
69	Duke	6	WCBC	Semi-dwarf / Susceptible
70	Mahigan	6	WCBC	Semi-dwarf / Resistant
71	Seebe	6	WCBC	Semi-dwarf / Resistant
72	Stetson	6	WCBC	Semi-dwarf / Intermediate
73	Tukwa	6	WCBC	Semi-dwarf / Intermediate

* WCBC: Western Canadian barley cultivars (Anonymous, 1989-2004).

Table 4-2. Single-spored isolates of *Rhynchosporium secalis* used in this study.

Isolate No.	Collection ID	Host cv./line	Isolate name	Location*
1	09-7	Duke	09-7 Duke	Edmonton (Ca.)
2	07-5	Harrington	07-5 Harr	Edmonton (Ca.)
3	B11-4	CDC Dolly	B11-4 Dolly	Beaverlodge (Ca.)
4	WRS 1860	CDC Guardian	WRS 1860	Olds (Ca.)
5	Lac-01	CDC Earl	Lac 2002	Lacombe (Ca.)
6	27-11	B1602	27-11 B1602	Edmonton (Ca.)
7	T155 (5)-1	RFLP Harrington	HarringT5-1	Toluca (Mex.)
8	T151 (1)-1	Harrington	HarringT1-1	Toluca (Mex.)
9	T162 (12)-1	TR251	TR251 T12-1	Toluca (Mex.)
10	T156 (6)-1	Stander	StanderT6-1	Toluca (Mex.)
11	T154 (4)-1	CDC Dolly 6	Dolly T4-1	Toluca (Mex.)
12	T158 (8)-1	CDC Dolly (P)	Dolly T8-1	Toluca (Mex.)
13	T163 (13)-1	CDC Dolly	Dolly T13-1	Toluca (Mex.)
14	T160 (10)-1	K8755	K8755T10-1	Toluca (Mex.)
15	T157 (7)-1	Zavila	Zavila T7-1	Toluca (Mex.)
16	T152 (2)-1	UNA 80	UNA80 T2-1	Toluca (Mex.)
17	T161 (11)-1	CI1240/Foma//CI16239.15D	CI T11-1	Toluca (Mex.)
18	T153 (3)-1	Seebe	SeebeT3-1	Toluca (Mex.)
19	Seebe-2	Seebe	Seebe-2	Toluca (Mex.)

* Ca. and Mex. represent Canada and Mexico, respectively.

Table 4-3. Reactions of barley scald differentials to nineteen single-spored isolates of *Rhynchosporium secalis*, where in a scale of 0-4, scores of 2.5 or greater were considered as susceptible reactions (S).

		Barley diferential cultivar/line								
Isolate No.	**Octal nomenclature**	**Osiris**	**Atlas**	**Turk**	**Trebi**	**Modoc**	**Brier**	**WW x G ***	**Atlas46**	**La Mesita**
1	0001									
2	0201				S					
3	1025		S				S		S	
4	0433			S			S	S		S
5	1535		S	S		S	S	S	S	
6	1011		S					S		
7	5737	S	S	S	S	S	S	S	S	S
8	1735		S	S	S	S	S	S	S	
9	1435		S	S			S	S	S	
10	5735	S	S	S	S	S	S	S	S	
11	1335		S		S	S	S	S	S	
12	1517		S	S		S		S	S	S
13	1635		S	S	S		S	S	S	
14	1733		S	S	S	S	S	S		S
15	1635		S	S	S		S	S	S	
16	1001		S							
17	1735		S	S	S	S	S	S	S	
18	1335		S		S	S	S	S	S	
19	5735	S	S	S	S	S	S	S	S	

* WW x G is an abbreviation for the barley differential Wisconsin Winter x Glabron.

Table 4-4. Pathotypes of *Rhynchosporium secalis* described by their octal name, number and percentage of susceptible genotypes, and disease index and aggressiveness.

		No. of susceptible[†]								
Pathotype	Iso	Differential	IC/CIM	WCBC	Total	%S	DI[‡]	*	Agg[§]	*
5735[a]	19	8	16	43	67	91.8	0.87	B	0.84	A
5735[b]	10	8	14	43	65	89.0	0.88	A	0.82	B
5737	7	9	13	43	65	89.0	0.84	C	0.79	C
1535	5	6	10	40	56	76.7	0.75	E	0.70	D
1335[a]	18	6	15	39	60	82.2	0.77	D	0.69	D
1635[a]	13	6	17	36	59	80.8	0.74	E	0.68	E
1335[b]	11	6	10	39	55	75.3	0.71	F	0.64	F
1735[a]	17	7	9	36	52	71.2	0.70	F	0.61	G
1735[b]	8	7	12	31	50	68.5	0.66	G	0.59	H
1517	12	6	10	33	49	67.1	0.67	G	0.56	I
1635[b]	15	6	5	33	44	60.3	0.65	H	0.54	J
1733	14	7	6	31	44	60.3	0.62	I	0.50	K
1435	9	5	6	30	41	56.2	0.60	J	0.47	L
1001	16	1	6	26	33	45.2	0.53	K	0.37	M
0201	2	1	6	29	36	49.3	0.45	M	0.37	M
0433	4	4	3	26	33	45.2	0.44	M	0.34	N
1011	6	2	5	21	28	38.4	0.46	L	0.32	O
1025	3	3	1	21	25	34.2	0.43	M	0.28	P
0001	1	0	2	6	8	11.0	0.18	N	0.10	Q

[a] and [b]: Pathotypes with the same octal name according to their reactions on the differentials were differentiated based on their reactions to the other 64 genotypes studied as indicated by the superscripts [a] and [b].

Iso: The previous ordered serial number (Isolate No.), where Iso 1-6 and 7-19 represent Canadian and Mexican isolates, respectively.

[†] Diff.: Differentials (out of 9); IC./CIM.: ICARDA/CIMMYT barley materials (out of 20); WCBC: Western Canadian barley cultivars (out of 73). %S: The percentage of susceptible reactions among the 73 barley genotypes tested

[‡] DI: Disease index (Salamati and Tronsmo, 1997).

[§] Agg: Aggressiveness (DI where disease score ≥ 2.5).

[*] Means with the same letter in each column are not significantly different based on Duncan's multiple range tests.

Table 4-5. Characterization of resistance in all 73 barley genotypes to 19 pathotypes of *Rhynchosporium secalis*.

Cultivars/lines	Summary of reaction[†]	No.S[‡]	DI[§]	Cultivars/lines	Summary of reaction	No.S	DI
Laurel	0000000000000000001	1	0.24	UNA80	0101111111101010101	13	0.68
Osiris	0000001001000000001	3	0.24	CDC Dolly	0010101111011111111	14	0.68
AC Hawkeye	0000001000000000101	3	0.26	Atlas46	0010101111111010111	13	0.69
Calicuchima.92	0000000000000000011	2	0.33	CDC Gainer	0000111111011111111	14	0.69
API/CM67-B//AGER	0000000000011101101	6	0.33	Bridge	0011111011111111111	16	0.69
CSBN 8527	0000001100001000000	3	0.34	Turk	0001101111011110101	12	0.70
Kasota	0000001001100000001	4	0.34	AC Bountiful	0111111101101010011	13	0.70
La Mesita	0001001000010100000	4	0.36	WW x G	0001111111111110111	15	0.71
AC Harper	0001001001010000100	5	0.36	Phoenix	1111101101111110001	14	0.72
Madre selva	0000001000001000010	3	0.37	TR139	0111101101100110111	13	0.72
Joso	0000110000011001100	6	0.38	CDC Dawn	0111101111101111111	16	0.72
Matico	0000001001001000111	6	0.39	Falcon	0001111111111111111	16	0.72
Gatillo-Bar	0000001101101000011	7	0.41	Bronco	1110101111111000111	14	0.72
Zarza	0000000101001101011	7	0.45	Manley	0110111111101110111	15	0.72
Zavila	0000101101111000011	9	0.45	B1215	0111101011111111111	16	0.73
CDC Richard	0000101101101000011	8	0.45	CDC Stratus	0110111111111111111	17	0.73
Manker/ATHS-B	0000100101101000111	8	0.47	TR145	0001101011111111111	14	0.73
Cardo	0000000001111100111	8	0.48	CDC Earl	0001101011111111111	14	0.73
Aleli	0000001101111000010	7	0.51	Atlas	0010111111111111111	16	0.74
Mahigan	0000001111110001111	10	0.51	CDC Guardian	0101111001111111011	14	0.74
Mejorana	0000101111110110011	11	0.53	Tukwa	0101101111111111111	16	0.75
Modoc	0000101101110100111	10	0.56	Brier (D)	0011101111101110111	14	0.76
Duke	0000111011000010101	8	0.56	CDC Fleet	0101101111111111111	16	0.76
CDC McGwire	0000101101111110011	11	0.60	Brier	0100111111111111111	16	0.77
Trebi	0100001101101110111	11	0.60	TR 251	0111111111111101011	16	0.78
K8755	1100101011111000011	11	0.61	AC Albright	0111111111111111111	18	0.78
AC Metcalfe	0100101001101000111	9	0.63	AC Rosser	0000111111111110111	14	0.80
Stetson	0100101001110110111	11	0.63	Stein	0101101111111111111	16	0.80
CI1240/Foma//CI16239.				CDC Sisler	0111101111101111111	16	0.82
15D	0101111111011001111	14	0.63	B1602	1101111111111111111	18	0.85
AC Lacombe	0110100111111000111	12	0.63	Duel	0111111111111111111	18	0.85
Leduc	0111111001111100111	14	0.63	Foster	0101111111111111111	17	0.86
Seebe	0000101111111001011	11	0.64	Harrington	1111111111111111111	19	0.88
Gloria-Bar/Copal	0100111101001111111	13	0.65	Stander	0111111111111111111	18	0.88
CDC Silky	0101101101101010111	12	0.65	Jackson	1111111111111111111	19	0.91
ORGE618	0100101111111011011	13	0.66	Cerise	1111111111111110111	18	0.91
AC Oxbow	0101111001110010111	12	0.66	Tercel	1111111111111111111	19	0.92

† Reaction to isolate 1 (left) to 19 (right) where 0 and 1 represent resistant and susceptible reactions, respectively.

‡ No.S: Number of susceptible reactions out of the total of 19.

§ DI: Disease index (Salamati and Tronsmo, 1997).

Table 4-6. Classification of barley genotypes using cluster analysis based on their reactions to the all isolates tested (disease index, DI).

Cluster (description)	Line[†]	Mean DI	St.dev[*]
I (Highly resistant)	Laurel, Osiris, AC Hawkeye, Calicuchima.92, API/CM67-B//AGER, CSBN 8527, Kasota, La Mesita, AC Harper, Madre selva, Joso , Matico	0.33	0.052
II (Resistant)	Gatillo-Bar, Zarza, Zavila, CDC Richard, Manker/ATHS-B, Cardo, Aleli, Mahigan, Mejorana, Modoc, Duke	0.49	0.047
III (Moderately resistant)	CDC McGwire, Trebi, K8755, AC Metcalfe, Stetson, CI1240/Foma//CI16239.15D, AC Lacombe, Leduc, Seebe, Gloria-Bar/Copal, CDC Silky, ORGE618, AC Oxbow, UNA80, CDC Dolly, Atlas46, CDC Gainer, Bridge,	0.65	0.031
IV (Moderately susceptible)	Turk, AC Bountiful (TR243), WW x G , Phoenix, TR139, CDC Dawn, Falcon, Bronco,Manley, B1215, CDC Stratus, TR145, CDC Earl	0.72	0.010
V (Susceptible)	Atlas, CDC Guardian, Tukwa, Brier(D), CDC Fleet, Brier, TR 251, AC Albright, AC Rosser,Stein, CDC Sisler	0.77	0.025
VI (Highly susceptible)	B1602, Duel, Foster, Harrington, Stander, Jackson, Cerise, Tercel	0.88	0.029

† WW x G: Wisconsin Winter x Glabron; Brier (D): The differential Brier.

* St. dev: Standard deviation.

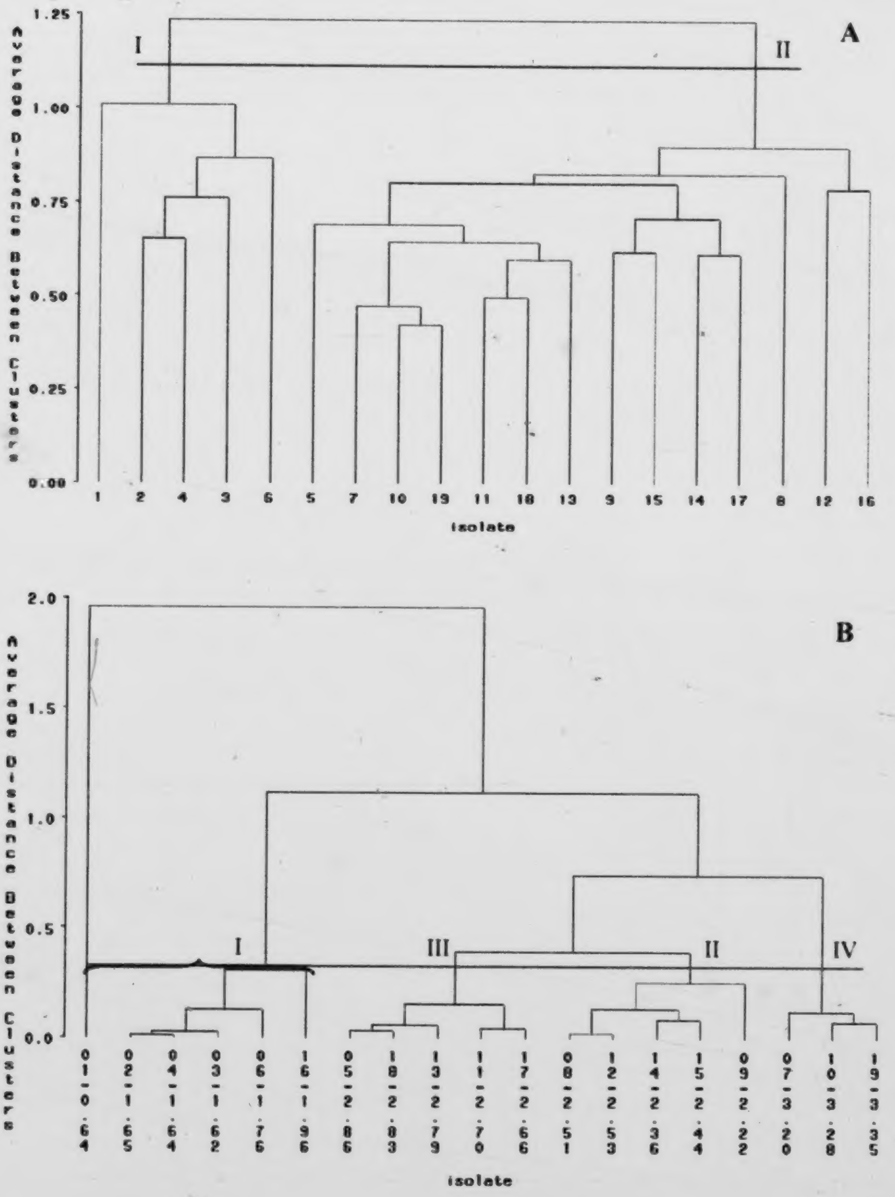

Figure 4-1. Hierarchical classification of 19 Canadian and Mexican isolates based on their pathogenic variation on a set of 73 different barley cultivars. A) Manhattan Metric was used as the measure of dissimilarity between the isolates (0 - 1.25 scale on the left side of the figure). B) The average reactions of the all barley genotypes to each isolate

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Chapter 5

Histopathological and physiological studies on barley leaves infected by *Rhynchosporium secalis*[§]

5.1. Introduction

The histology and biochemistry of interactions between *Rhynchosporium secalis* (Oud.) J. J. Davis, the causal agent of the scald disease, and its host, barley (*Hordeum vulgare* L.), have been much investigated (Able, 2003; Ayesu Offei and Clare, 1971; Caldwell, 1937; Gierlich *et al.*, 1999; Jones and Ayres, 1974; Jörgensen *et al.*, 1993; Lee *et al.*, 1999). Various studies have been undertaken to elucidate the infection process of the fungus and the reaction of its host (Ali, 1974; Ayesu Offei and Clare, 1970; Doken, 1988; Steiner-Lange *et al.*, 2003; Xi *et al.*, 2000). The barley-scald pathosystem has also been investigated to determine the chemical and enzymatic factors involved in the pathogenesis of *R. secalis* and the resistance mechanisms of the host (Jones and Ayres, 1974; Olutiola and Ayres, 1973; Peltonen, 1995; Tewari, 2000). Tewari (2000) investigated the potential role of calcium as a resistance-related factor and demonstrated that the fungus sequesters calcium from the host tissue during pathogenesis, by production of different organic acids. Energy dispersive X-ray microanalyses (XRM) in conjunction with scanning electron microscopy (SEM) have been shown to be a useful means to determine elemental changes associated with disease resistance (Kim *et al.*, 2002; Sugawara *et al.*, 1998; Williams *et al.*, 2002). Silicon (Si^{4+}) has been shown to benefit plants in a number of ways including normal growth and development, and through increasing resistance to fungi and other parasites (Epstein, 1999; Kim *et al.*, 2002). Silicon has been found to be present mainly in the epidermal cell walls, middle lamellae, and intercellular spaces within the sub-epidermal tissues (Kim *et al.*, 2002). Several activities as a pre-existing or dynamic defense resistance factor and its contribution to increase mechanical resistance of injured tissue by silisification have been alluded (Agrios, 1997; Epstein, 1999; Elahinia, 2000a, b; Kim *et al.*, 2002; Zeyen *et al.*, 2002). Silicon is mobilized at the points of penetration by *R. secalis* on barley and the areas enriched in Si are larger in the resistant than in the susceptible cv. (Turkington *et al.*, 1999). Calcium (Ca^{2+}) ion is considered to be a component of signaling pathways; and potassium (K^+) and sodium (Na^+) ions are directly involved in regulation of intracellular osmotic pressures (Gelli *et al.*, 1997; Karley *et al.*, 2000a; Karley *et al.*, 2000b). In recent years, it has become evident that mineral distribution in barley leaves is highly heterogeneous (Karley *et al.*, 2000b). There has been only little microanalytical work on the barley-scald pathosystem.

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When a barley plant is challenged by an isolate of the scald pathogen, the compatibility of interaction is primarily dependent on their genotypes, and the presence of corresponding resistance gene(s) in the plant and avirulence gene(s) in the fungus, in particular (Able, 2003). In an incompatible interaction, where a resistance gene in the plant corresponds to an avirulence gene in the pathogen, no visible sign of disease is observed (Ali, 1974). However, the plant may undergo several molecular and ultrastructural changes to resist the pathogen. Xi *et al.* (2000) described the formation of cell wall apposition at the site of penetration by the scald fungus as a resistance response. Apposition and halo formation are structural responses of plants that may confer resistance against pathogen by establishing a penetration barrier. Accumulation of calcium in the epidermal cells has been reported to be associated with the formation of halos around the points of penetration of the fungus (Mukerji and Tewari, 2000). Mukerji and Tewari (2000) suggested that calcium mobilization at the points of challenge by the pathogen might play a role in host resistance in the barley-scald pathosystem. Tewari (2000) detected different calcium-containing crystals which were associated with mobilization of calcium in resistant barley tissues upon challenge by *R. secalis* (Tewari, 2000). While recent work on the barley-scald pathosystem indicates that the hypersensitive response does not occur in incompatible interactions with *R. secalis* (Able, 2003; Steiner-Lange *et al.*, 2003), the formation of small dark-brown patches on the leaves where infection has been unsuccessful, was referred to as this type of resistance

(Ayesu Offei and Clare, 1971). The pathogenicity-related proteins have been reported to play a role in scald disease resistance (Hahn *et al.*, 1993; Zareie *et al.*, 2002).

In compatible reactions to *R. secalis* which lead to development of typical lesions of scald, a plant may either undergoe complete compatibility without resisting as in the fully susceptible plants, or implement some reactions to reduce the damage caused by the invading fungus, as in genotypes with intermediate levels of resistance. The phenomenon of slow-scalding is of the latter type of reaction where a compatible reaction is coupled with the slow progress of disease over the course of epidemic (Sorkhilalehloo *et al.*, 2001). The slow-scalding lines exhibit lower rates of disease severity (e.g. lesion area/lesion number) and consequently suffer less damage to the photosynthetic area by the disease. However, there is concern as to whether these few lesions cover the leaf base area (auricle), through which all necessary materials for the plants are conducted. An auricle could trap both splashed scald spores and run-off water (rain droplets), and hence, it could provide ideal conditions for the formation and development of scald lesions. However, distribution of scald lesions on plant tissue is not likely to follow any specific pattern, and can happen anywhere on the leaf surfaces.

The leaves of barley are the main targets of the scald pathogen. Each leaf consists of three major tissues i.e. single-layer epidermis, mesophyll, and vascular bundles (Esau, 1965). The epidermis is the first barrier to the attack by plant disease-causing agents. The necrotrophic fungus, *R. secalis*, penetrates the leaf's uppermost layers and initially develops extra-cellularly beneath the cuticle without formation of haustoria (Lehnackers and Knogge, 1990). Little information is available as to how scald may affect the vascular bundles and its major components, xylem, and phloem. Collapse of epidermal cell walls, tissue necroses, and xylem-vessel plugging were observed as histopathological changes induced by the phytotoxic glycoprotein (Auriol *et al.*, 1978). The xylem and phloem are the principal water/mineral- and food/photosynthetic material-conducting tissues in vascular plants (Dickison, 2000). The effect of disease on the structure and function of auricle at the leaf base as a major water- and food- conducting area of the leaf has neither been examined microscopically nor physiologically so far. Collection of vascular exudates from pea seedlings and detached leaves of a *Hordeum* species using ethylenediaminetetracetic acid (EDTA) has been shown to be a useful technique for characterization of the content of phloem sap (Hanson and Cohen, 1985; Weibull, 1994).

This study was undertaken (1) to compare the infection processes of the scald pathogen in resistant, slow-scalding, and susceptible cultivars of barley using scanning electron microscopy (SEM); (2) To determine the levels of calcium, potassium, silicon, and sodium in barley leaves before and after inoculation in both compatible and incompatible interactions, using SEM in conjunction with energy-dispersive X-ray microanalyses (XRM), and to characterize disease/resistance-related factors such as crystal formation; (3) to study histological changes in the host vascular bundles in compatible reactions with the scald pathogen under different disease severities using light microscopy (LM) and transmission electron microscopy (TEM). Such studies could reveal if transportation of water and photosynthetic materials through an infected auricle is altered upon infection; and (4) to compare the transportation of vascular exudates through healthy versus diseased auricles, following collection of vascular exudates using EDTA.

5.2.Materials and methods

5.2.1. Plant materials

For the infection study and X-ray microanalysis, two scald susceptible barley cvs., Stander and Jackson, two ICARDA/CIMMYT slow-scalding lines, Zavila and UNA 80, and one resistant differential, Osiris, were grown under indoor conditions suitable for scald seedling evaluations (Chapter 2). At the four-leaf stage, the second leaves of seedlings exhibiting scald lesions together with healthy samples of each cultivar were studied.

To compare reactions of Stander, Osiris, UNA 80, and Zavila at the adult-plant stage, these genotypes were grown under indoor conditions and their penultimate leaves were inoculated with one isolate of scald at ZGS 33 (Zadoks *et al.* 1974). Auricle infection was also studied on flag and penultimate leaves of Stander after anthesis.

5.2.2. Inoculation

Seven different scald isolates i.e. Iso 5, Iso 7, Iso 11, Iso 13, Iso 14, Iso 18 and Iso 19 belonging to Dr. J. P. Tewari's barley pathogen collection at the University of Alberta were used to artificially inoculate the seedlings at the 2-leaf stage. The final concentration of inoculum was adjusted to 1×10^5 conidia/ml. Details about these isolates are given in Chapter 4 and the inoculation technique is described in Chapter 2. For the infection study, only seedlings inoculated with Iso 13, and adult-plants inoculated with Iso 5 were studied, whereas for XRM all available fungal and plant materials were tested. The leaf base area (auricle) of the penultimate and flag leaves of the cultivar Stander was also inoculated with Iso 5 at the end of the stem elongation stage by placing a small piece of the scald culture grown on lima bean agar (LBA, Chapter 2) onto the auricles, spore-side down (Habgood, 1977).

5.2.3. Disease assessment

Seedlings were scored at the 4-leaf stage according to the 0-4 scale (Chapter 2), where scores ≥ 2.5 represented susceptible reactions. In the auricle study, the leaf base was arbitrary scored to reflect relative severity of scald at the point of infection as follows: 0, healthy auricle; 1, low to intermediate; 2: intermediate to severe; and 3, severe, with the whole leaf base scalded, 4, very severe, where leaf base tissues were partially macerated around the main vein.

Right after disease assessment, healthy vs. inoculated leaf samples were examined using LM, SEM, XRM, or sugar/protein assays.

5.2.4. SEM and XRM

The samples were examined using SEM (Hitachi S-2500, Tokyo) and XRM (JSM 6301-F equipped with IMIX-PST, PGT, Princeton, NJ). The SEM samples were prepared using a standard protocol similar to that explained by Green *et al.* (2001). The samples (4-5 mm^2) were fixed in 2.5% glutaraldehyde in cacodylate buffer for 1 h followed by 3 washings with the same buffer. The tissues were then post-fixed in 2% (v/v) osmium tetroxide in cacodylate buffer for 3 h. Following a further washing in ddH_2O, the leaf pieces were dehydrated in an ethanol-propylene oxide series. The preparation of samples for the infection study was according to a critical point drying procedure where a critical point-drying at 31°C was applied to the samples for 5-10 min. In contrast, the samples for XRM were initially air-dried at 25°C for one week. Prior to analyzing the plant materials with SEM/XRM, the samples were mounted onto stubs using a double-sided carbon tape, and then sputter coated with gold (model Semprep 2, Nanotech, Cambridge). Using a Link eXL energy-dispersive X-ray system with a light element detector, calcium (Ca), silicon (Si), potassium (K), and sodium (Na) levels at the surface layers of the epidermis of different barley leaf samples were then studied at a magnification of X100 and an accelerating voltage of 20 keV.

5.2.5. Auricle study

5.2.5.1. Light microscopy and transmission electron microscopy

At the milk development stage, leaves of the cultivar, Stander exhibiting different levels of disease severities only at their auricles were cut 2-3 mm below their auricles and freshly placed into distilled water for 15 minutes. Uninoculated samples were also included in the auricle study as healthy checks. Pieces of transversely sectioned auricle were then stained with the Lactophenol dye and examined by LM. The same samples were ultra thin-sectioned and studied by TEM (Hitachi H-7000, Tokyo). The materials for TEM were prepared using the same method of Hargreaves and Keon (1983). The leaf tissues (2-3 mm^2) were pre-fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.2) at room temperature for 1 h. After washing with the same buffer (3X), the tissues were post-fixed in 2% OsO4 in cacodylate buffer for 3 h, followed by a further washing in ddH$_2$O, and dehydrated in an ethanol-propylene oxide series. The samples were then transferred into Araldite CY212 mixture/Propylene oxide for 1 h and embedded in complete Araldite CY212 mixture for over-night in a vacuum desiccator. Polymerization at 60°C for 2 d, and cutting the samples to ultra-thin section were the last two steps before examining the leaf tissues by TEM.

5.2.5.2. Exudate collection and analyses techniques in conjunction with protein and sugar assays

For collection of vascular sap exudates from both the healthy and diseased samples, the entire detached leaves were placed into a transparent glass tube containing 200 µl of 0.5 mM EDTA, pH 7.5, and kept in a sealed humid polyethylene sheet tent at 25°C., and light intensity 15 klx for 2 hours. The extracted exudates were immediately frozen at -20°C and stored for use in the protein and sugar assays.

The amount of total protein in 10 µl of each sample was determined using the method of Bradford (Microtiter plate protocol, Bio-Rad protein assay, BIO-Rad, Hercules, Calif., U.S.A.) where BSA (1 mg protein/ml) was used as reference protein. The extracts were assayed on the Microtiter plate (Costar, Corning, N.Y., U.S.A). The samples were then placed in a Kinetic microplate reader (Spectra MAX 190, Molecular Device UV max, Sunnyvale, Calif., U.S.A) and the amount of protein for each specimen was measured at 750nm absorbance after 15min.

Sugar assays in the exudates were done by high performance liquid chromatography (HPLC). The sugar assays were conducted using 100 µl of the exudates on a Varian 9010 pump with a Hewlett Packard 1050 autosampler and an Alltech 5000 (ELSD) at 125°C, and a nitrogen gas flow of 3.0 SLPM. Peaks were integrated using Shimadzu Class-VP 4.2 software. Sugars were separated on a Supelcosil LC-NH2 5 µm column (4.6 mm x 25 cm) using a water: acetonitrile gradient.

5.2.6. Traits and statistical analysis

For the infection study, latent period was recorded for both seedling and adult-plant stages as the number of days between the inoculation and the appearance of the first lesion on the plant leaf tissues. Sporulation proportion was recorded as the number of samples in which sporulation was observed out of the 6-9 samples studied. Using a haemocytometer, conidia washed from pieces of leaf tissues (10 mm) bearing a lesion into 2 ml of water were counted as the number of spores per lesion. In addition to the latent period, the data on sporulation of the plant genotypes were analyzed using a completely randomized design (CRD) with three replicates. Tukey's multiple mean tests were performed to statistically compare the data.

The levels of Ca, K, Si, and Na as the percentage of gold used as coating on the surface of leaf samples were used to compare the elemental changes in the barley genotypes with different levels of resistance to the scald pathogen before and after inoculation with various isolates of *R. secalis*. The X-ray microanalysis data were statistically analyzed with two different models for analysis of variance: (A) Nested two-way analysis of variance with three replicates where isolate (Iso) was nested within genotype (Gen), (B) Factorial experiment with three replicates. where Iso X Gen interaction was of particular interest. The effects of Gen and Iso treatments within genotypes, Iso (Gen) were determined using the nested design where 32 different combinations of genotypes and isolate treatments were studied in 3 replications. On the other hand, the factorial analysis was used to study the interaction between Iso and Gen (Iso X Gen), where a balanced set data were analyzed for XRM on four genotypes challenged with four isolates of *R. secalis*. Gen and Iso were considered as fixed factors. Tukey's pairwise LS-mean test was used to check the significance of differences among genotypes/isolates for the traits studied. For the isolates Iso 5, Iso 11, and Iso 13, Pearson's coefficients of correlation were calculated to study the relationship between the disease score and the level of the elements in the genotypes within isolates (n = 24).

Analyses of variance for the infection and XRM studies were performed using Proc GLM (SAS Institute Inc., 1989).

5.3. Results

5.3.1. Infection study

Table 5-1 shows disease characteristics of barley genotypes challenged with Iso 13, and Iso 5 at seedling, and adult-plant stages, respectively. Significant differences were observed among genotypes studied in the infection study. Latent period, lesion type and sporulation in the resistant barley differential, Osiris were significantly different from both the susceptible and slow-scalding genotypes. The susceptible genotypes did not show resistance to any of the infection process's components, and hence exhibited a fast process of infection characterized by their shorter latent periods at both seedling and adult-plant stages. In general, the two slow-scalding lines exhibited similar reactions to the infection processes and were different for the traits studied as compared with the resistant or susceptible genotypes except for their sporulation at the seedling stage where no differences could be observed among genotypes with compatible reactions to *R. secalis*. Except for Osiris with no sporulation, normal sporulation was observed in the majority of diseased samples studied at the seedling stage. However, at the adult plant stage, only about one-half of the slow-scalding samples showed sporulation where the amount of conidia formed on a lesions were significantly higher in Stander rather than in the slow-scalding genotypes.

As revealed by SEM, the susceptible cvs. Stander and Jackson, exhibited normal conidial germination, appressorium formation, massive subcuticular mycelium development, and abundant sporulation (Figure 5-1A and 5-2). The germination of conidia on resistant and slow-scalding genotypes and subsequent steps in the formation of germ tubes and infection pegs, and penetration looked quite similar to that of susceptible genotypes (Figure 5-1B and D). In the slow-scalding lines, the rate of growth of subcuticular mycelia and sporulation seemed to be slightly lower as compared with those of the susceptible cultivars. However, technically these differences could not be measured, and hence, statistically could not be proven. Sparse subcuticular mycelium development coupled with no sporulation was observed in the cultivar Osiris, whereas morphologically Osiris was observed to be immune with no symptom of the scald disease. In contrast, both the susceptible and slow-scalding lines showed typical lesions of scald where the former exhibited large coalescing lesions of scald as compared with those of the later which were discrete. Contrary to both resistant and slow-scalding genotypes, secondary infections were observed in all susceptible samples as characterized by infections (on areas close to the margin of lesions) caused by conidia which were produced during the first cycle of disease. As shown in Figure 5-1D, attempted penetration characterized by a very long germination tube coupled with no sign of appressorium formation was observed on slow-scalding samples.

5.3.2. SEM in conjunction with XRM

Scanning electron micrographs of barley leaf surfaces inoculated with *R. secalis* showed various shapes of Ca/K-containing crystals associated with the reactions of plant cells upon infection (Figures 5-3 and 5-4). Figure 5-3 shows crystals formed on the margin of infected area where subcuticular mycelia were most likely absent. Micrographs 5-3A and 5-3B show crystals containing K beneath the epidermal cell wall (UNA 80 x Iso 18: resistant reaction), and containing Ca emerging through the cuticular layer (UNA 80 x Iso 19: susceptible reaction), respectively. Micrographs 5-3C and 5-3D show the crystals containing Ca both beneath the epidermal cell wall (Stander x Iso 14: susceptible reaction) and on the leaf surface (Stander x Iso 18: susceptible reaction), respectively. Figure 5-4 shows crystals formed on top of the subcuticular mycelia. Micrographs 5-4A to 5-4D show plate-like crystals and druses thereof containing potassium/calcium (Stander x Iso 13: susceptible reaction), bipyramidal crystals containing calcium/silicon (Osiris x Iso 13: resistant reaction), druses containing calcium/potassium (Zavila x Iso 7: susceptible reaction), and raphide druses containing calcium (UNA 80 x Iso 19: susceptible reaction), respectively. Figure 5-5A to D, show the XRM spectra for crystals which were shown in Figure 5-4A, D, C, and B, respectively. The dominant peak in Figure 5-4A is for K, and other notable features are peaks for Ca and S where druses were found on top of the subcuticular mycelia growing on the leaf surface of Stander. Microanalyses of the crystals observed in Figure 5-4B indicated high levels of Ca and Si in the epidermis of Osiris in its resistant reaction with *R. secalis*. Calcium was commonly detected at very high levels in slow-scalding lines (Figure 5-4C and D) where druses containing calcium/potassium and calcium/phosphorus were found in compatible reactions of Zavila and UNA 80 (Figure 5-5C and B), respectively.

As shown in Table 5-2, the XRM data were analyzed using two different statistical models in which the effects of genotypes (Gen), isolate treatments (healthy vs. inoculated with different isolates of scald), and their interaction (Gen x Iso) were estimated. Table 5-2A shows the results of XRM for a nested design where 32 different combinations of genotypes and isolate treatments were studied in 3 replications in order to determine the effects of Gen and isolate treatments within genotypes, Iso (Gen). Table 5-2B represents the results of a study where lesser number of genotypes and isolate treatments were analyzed, but, the effects of isolates and Gen x Iso interaction could be separately studied. In all cases, significant differences were observed among the traits/treatments studied. The reactions of barley seedlings to the attack of scald isolates, which were presented as "score", were significantly different indicating the differential responses of genotypes to different inoculation treatments. Mean disease score, standard deviation, and groupings of all genotypes are given in Table 5-3. As shown in this table, except for a few cases, all susceptible and slow-scalding genotypes showed compatible reactions with the scald isolates whereas Osiris reacted as being resistant to all the 3 isolates to which it was challenged with.

The genotype and isolate treatments were also different in all the studied traits. The mean/LS-mean tests of the nested design and factorial experiment are shown in Table 5-4 and Table 5-5, respectively. Table 5-4A and 5-5A show that there were significant differences among the genotypes in terms of the elements studied. This could also be concluded from Figure 5-6. Comparing the average level of elements studied in Stander as a susceptible check with those of the other genotypes, Osiris as the most resistant cultivar showed higher levels of K and Ca. The highest levels of Na were detected for UNA 80 and Osiris. Osiris also showed the minimum level of Si in the surface of epidermis whereas Si for the rest of barley genotypes was not significantly different. In contrast, no specific pattern was observed for these elements in order to distinguish between the susceptible and slow-scalding genotypes. Zavila and UNA 80 showed different levels of K and Na, where their amounts of Ca and Si were not significantly different. Stander had similar K and Na levels as compared to those of Zavila (Table 5-5A). UNA 80, on the other hand was similar to Jackson for the levels of K, Ca, and Si (Table 5-5B).

Significance was observed among the isolate treatments for a given element within each genotype (Table 5-4B). The uninoculated treatment (healthy samples) showed statistically the lowest levels of the elements, K, Ca, and Si within each genotype. However, Na did not show such a pattern. Also high levels of this element were seen in the healthy samples of cvs. Osiris UNA 80, and Jackson. These indicated changes in levels of these elements upon challenge with the scald isolates. However, these changes were necessarily observed for all the isolates testes. For example, the level of Ca in uninoculated samples of Osiris was only significantly different from that of inoculated with Iso 13. Also there were no differences among the isolate treatments of Osiris for the element, K. To get a general view of the effects of different isolates on the elemental changes, inoculation treatments were also compared according to the results of factorial experiment (Table 5-5B). As shown in Tables 5-4B, and Figure 5-5B, again the levels of elements analyzed on the surface of specimens were different before and after inoculation/infection. Among the isolates studied, Iso 13 seemed to cause maximum elemental changes in the plant after inoculation. Iso 11 reacted similarly whereas the samples inoculated to the Iso 5 had significantly different amounts of elements as compared with those inoculated by Iso 13 and Iso 11.

As shown in Table 5-2B, Gen x Iso effects were also found to be significant for the elements studied. The presence of such interactions indicated that not only the differential responses of a given genotype to different isolate treatments could be present, but also there could be differences among genotypes in terms of the extent of changes in their levels of elemental compositions under different inoculation treatments. These are presented in Figure 5-7, indicating changes in the barley epidermal cell's Ca, Si, K, and Na before and after inoculation with different scald isolates Except for Zavila and UNA 80 on the left side of the graphs, all other genotypes were inoculated with the scald isolates Iso 13 (left) and Iso 11 (right). Osiris exhibited resistant reactions to the isolates, Iso 11 and Iso 13. Also, as shown in Table 5-3, Zavila and UNA 80 on the left side of the figure were resistant to Iso 14 and Iso 18, respectively. As shown in Table 5-7, depending on the isolate used, there were significant changes in the elemental composition of a genotype after inoculation. Also the rank and order of genotypes changed depending on the element and different types of inoculation treatment. The rates of these changes were also different while using different isolates of scald. A general increasing trend was observed for Ca, Si, and K in both compatible and incompatible interactions whereas Na, in most cases, showed decreasing trends after inoculation. The level of Ca was higher in Osiris once inoculated with Iso13 (score 0.3±0.4) than when inoculated with Iso 11 (score 1.7±0.5). Calcium levels in compatible reactions of UNA 80 and Zavila showed higher increases as compared to those in incompatible interactions. Calcium levels in Stander after inoculation with Iso 11 and Iso 13 showed two different trends as Ca did not change significantly after the later treatment but increased after the former. Jackson, on the other hand, had similar increase in its Ca level after inoculation with both isolates. The increasing levels of Si were also observed in both resistant and susceptible interactions. Zavila and Jackson showed an increasing level of Si upon inoculation with Iso 11 where

Osiris and UNA 80 showed the least increase in their Si levels. Potassium had its maximum levels in Osiris; however, it did not differ significantly upon inoculation. Similar trends were observed for Zavila whereas UNA 80 and Jackson showed significant increases in their levels of K after inoculation in both compatible and incompatible reactions. Conversely, UNA 80 and Jackson, while showing the highest levels of Na, showed significant decreases after inoculation. Interestingly, a general decreasing level of Na was observed in most studied samples upon inoculation. The results indicated that changes in the levels of these elements might be inter-related.

As shown in Table 5-6, depending on the isolates used, significant level of associations was seen among the elements studied and the disease scores. Pearson's coefficients of correlation between Ca and disease score were all positive indicating that Ca was associated with higher scores of disease. However, only for Iso 11, this coefficient was significant. The correlation between Na and K in all cases were negative and non-significant. Also, Ca was observed to significantly and positively correlate with the levels of K in all the isolates studied. For Iso 11, Si was positively and significantly correlated with disease score and Ca, whereas there was negative and significant coefficient of correlation between disease score and Na. Disease score in Iso 13 was positively and significantly correlated with K and Si.

5.3.3. Auricle study

LM and TEM studies were undertaken to determine if and how the fungus could affect the leaf tissues at different disease severities, and interact with the vascular cells. Figure 5-8A to 5-8C show the healthy to heavily infected auricles, respectively. Complete infection on the sheath of a barley plant is also shown in Figure 5-8D. Both susceptible and slow-scalding lines set high levels of auricle infection since the leaf bases serve as suitable areas for trapping both the scald conidia and droplets of water. Morphologically, for the disease score of 0 to 3, it was clearly observed that the infected samples exhibited a green leaf with no signs of chlorosis/necrosis through the whole leaves except for their auricles. However, at the disease score of 4, where the auricles were partially macerated, the vascular bundles were expected to be heavily damaged.

LM and TEM

Figure 5-9 shows transverse sections of the barley leaf auricles showing varying levels of damage caused by *R. secalis* (micrographs A, B, C, and D represents barley leaf auricle samples with disease scores of 0, 3, 3, and 4 out of 4, respectively). The mesophyll and vascular tissues looked unaffected in both healthy and diseased samples with disease scores of ≤ 2. Samples with higher disease scores showed greater degrees of maceration on their epidermal and mesophyll cells. In most samples studied with a disease score of 3, the inner layer of the bundle sheath remained unaffected, providing the xylem and phloem cells with a good level of protection (Figure 5-9, micrographs B and C). Figure 5-9D represents a sample with a disease score of 4, where even phloem and xylem tissues were partially macerated. The interaction of barley and the scald pathogen was also studied in different tissues using TEM (Figure 5-10). Apparently, the fungus did not enter any living cell. In comparison with a healthy sample (Figure 5-10A), auricle samples with a disease score 1 or 2 (Figure 5-10B) only showed subcuticular invading hyphae in their epidermal cells and no fungal mycelium was found in contact with their mesophyll cells. Degraded epidermal cells were observed in some samples with a disease score of 2. Auricle samples with disease scores of 3 (Figure 5-10C and D) and 4 (Figure 5-10E); however, showed their mesophyll and bundle sheath cells in direct contact with the fungal mycelia. The epidermal cells in samples with disease scores of ≥ 3 were partially to completely destroyed.

Exudation technique in conjunction with protein and sugar assays

As shown in Figure 5-11, protein and sucrose transport were severely impaired only in specimens with a disease score of 4 where phloem cells were partially macerated. Within each disease score, protein levels of healthy and diseased auricles were not significantly different except for the disease score of 4 where no protein was detectable in the exudates. For sugar assays, standards were used to detect fructose, glucose, sucrose, maltose, maltitriose, turanose, lactose, lactulose, palatinose trehalose, and raffinose using HPLC. However the only detectable sugar was sucrose, which was present in all diseased and healthy samples. The results obtained from the sugar assays were similar to those for protein assays indicating that there were no significant differences among diseased (with disease scores of ≤ 3) and healthy samples. Therefore, the vascular bundle in the score of ≤ 3 was considered to function well in transporting vascular sap materials. With respect to sensitivity of the techniques, it could be stated that the other sugars either did not exist in the samples studied or their amounts were less than 40 µg/ml. With respect to the sensitivity of the protein assay, it should be noted that any level below 50 µg/ml was detectable in the technique applied. Therefore, the amounts of sugar and protein in the leaf samples with a disease score of 4, if not zero, was less than 40 and 50 µg/ml, respectively.

5.4. Discussion

5.4.1. Infection study

The infection process of *R. secalis* was compared for a set of resistant, slow-scalding, and susceptible barley genotypes. Various stages of infection and sporulation were observed in susceptible reactions. This was in accordance with prior literature studying susceptible reactions to scald (Ayesu Offei and Clare, 1970; Caldwell, 1937). Despite the fact that no typical lesion was found on the resistant scald differential genotype, Osiris, SEM study showed sparse subcuticular development of the fungal mycelia coupled with no sporulation. Jones and Ayres (1974) also reported that the subcuticular hyphae of *R. secalis* were sparse in the resistant reaction whereas Jörgensen *et al.* (1993) pointed out that in the resistant interaction, only restricted sporulation occurred at the leaf margins of seedlings. It has also been stated that papilla formation could serve as being a resistance mechanism to prevent penetration and hence retard disease development (Jörgensen *et al.*, 1993; Xi *et al.*, 2000). In this regard, Xi *et al.* (2000) also indicated that the mechanisms responsible for resistance are likely characterized as penetration prevention rather than as a slow rate of mycelial growth after successful penetration. The aforesaid differences in resistant reactions may reflect various mechanisms of resistance in different barley genotypes or interactions with various pathotypes of the scald fungus. The slow-scalding lines also showed compatible reactions with the scald pathogen at seeding stages similar to those in the susceptible cultivars. However, they seemed to prevent a fast disease development by reducing some components of infection cycle, as they showed longer latent periods, less frequent and smaller lesions, lower rates of sporulation, and resistance against secondary infections. A longer period between inoculation and sporulation could play a crucial role in the slow-scalding type of resistance by reducing disease progress. Hence, slow-scalding lines with longer latent periods are expected to exhibit disease symptoms later and undergo fewer disease cycles during the course of epidemics. Less severity of disease could also be due to the formation of fewer and smaller lesions as compared to those of frequent and overlapping lesions in the susceptible genotypes. As mentioned before, attempted penetration characterized by a long germination tube coupled with no sign of appressorium formation were considered as signs of suppressed secondary infection in Zavila and UNA 80.

5.4.2. X-ray microanalysis

Scanning electron micrographs and XRM spectra of barley leaf surfaces inoculated with different isolates of *R. secalis* showed Ca/K-containing crystals of various shapes. No such crystals were found on the healthy/uninoculated samples at all. Analyses of certain inorganic elements (Ca, Si, K, and Na.) in the superficial layers of the plant tissues showed that the levels of these elements in uninoculated samples differed significantly after inoculation. It was also shown that the extent of changes in the elemental composition of epidermal cells is dependent on the genotypes and interactions with scald isolate(s). The data could support the hypothesis of the presence of an active mechanism(s) of response in the plant to fungal invasion associated with resistance and/or pathogenicity in the barley-scald pathosystem, which could reflect mobilization of elements in superficial layers in the plant tissues or their sequestration by *R. secalis*. Calcium and potassium were commonly detected at very high levels in the crystals/druses observed in slow-scalding lines, Zavila and UNA 80 similar to those observed in the susceptible cultivar Stander. The production of druses containing calcium in a compatible barley-scald interaction was a good indication of calcium sequestration from the host cell walls by the pathogen as a result of *R. secalis* infection, and is in agreement with the previous work by Tewari (2000). The crystals were also found on the surface of epidermis emerging through the cuticular layer associated with resistance response to infection. For Osiris, bipyramidal crystals containing calcium/silicon on the subcuticular mycelia were likely formed directly as a resistant reaction to reduce the production of stroma and to prevent subsequent sporulation. The formation of this type of crystals might also function to reduce the severity of scald in the slow-scalding lines. Similar conclusions can be drawn for the susceptible genotype, Stander which showed such druses formed on the subcuticular mycelia of scald through its epidermis.

The cultivar Osiris showed a significantly different elemental composition from the other genotypes studied, whereas no particular differences were evident between slow-scalding and susceptible lines. This would suggest that the elemental compositions of Osiris might be related to its unique resistance features. We discussed that Osiris has also shown a high level of stability over different locations and years (Chapter 3 and 4).

The correlation study also revealed that increases in the levels of Ca and Si which were associated with higher scores of disease, could indicate the sequestration of inorganic elements. However, the elevation of these elements in epidermal cells upon inoculation could indicate their mobilization by plant cells. The negative correlation between Na and K could be due to a negative shift between their ion currents in barley cell membrane upon infection. A possible role for ion channels which might be involved in plant response to the attack of a pathogen e.g. signal transduction by the plant cells, may make it worthwhile to study the barley-scald pathosystem from this perspective using a patch-clamp technique. This technique has been frequently used to study the effects of cation channels on the activation or inhibition of different physiological phenomena in barley (Amtmann *et al.*, 1997; Hedrich *et al.*, 1990). Further investigations on resistance and pathogenicity mechanisms in the barley-scald pathosystem are required to interpret the results obtained.

5.4.3. Auricle study

Auricles and sheaths are two important passages in a barley plant where water, minerals, and photosynthates are transported. Scald infection in these areas could seriously affect the transportation of materials which are necessary for the growth and development of a plant. It was previously suggested that the auricle infection while covering about 5% of the leaf area, could cause enough damage to the phloem so that

100% of the leaf area should be considered as being infected (personal communication to Prof. J.P. Tewari from late Prof. W.P. Skoropad, University of Alberta). We hypothesized that if *R. secalis* could damage the vascular bundle sheath or plug it with phytotoxic materials as reported by (Mazars *et al.*, 1989), the consequences could be serious. We used the EDTA technique to study the effects of auricle infection on transportations of materials through the vascular bundle. EDTA could chelate many chemicals such as sugars, amino acids, and metal ions. Morphologically, for disease scores of 0 to 3, it was clearly observed that the infected samples exhibited a green leaf with no signs of chlorosis/necrosis through the rest of the leaves except for their auricles. Since water and minerals are conducted through the xylem tissues, it was clear that the xylem tissues most likely remained unaffected under these circumstances. It was also revealed that within each score of 1-3, there were no significant differences between transport of protein and/or sugar through healthy and infected auricles. However, samples with higher disease scores showed greater degrees of maceration in their epidermal and mesophyll cells. Protein and sucrose transports were only severely impaired in specimens with a disease score of 4 where phloem cells were partially macerated. Hence, histological and physiological studies have only partly validated the concept that auricle infection may adversely affect transport of photosynthates from the leaf into the plant. We suggest considering this fact in scoring of plants with high rates of severe auricle infections, even if the scalded area only accounts for 5-10 percent of the total leaf blade.

In this study, only the dead-looking cells were seen to be colonized by the fungus. Degradation of the epidermis cell walls by the pathogen will be required before accessing the mesophyll cells. Ayesu and Clare (1970) described that the death of mesophyll cells occurred before being reached by the pathogen. The authors also mentioned that only dead/dying cells were colonized by the hyphae. However, we observed that the fungal mycelia interacted superficially with apparently living mesophyll and bundle sheath layer cells. With respect to plant response in a compatible reaction to the invasion of *R. secalis*, our results are in accordance with the findings of previous workers (Jörgensen *et al.*, 1993; Xi *et al.*, 2000).

5.5. Tables and figures

Table 5-1. Disease characteristics for barley genotypes tested in the infection study. Note that according to Tukey's multiple mean's tests, means with the same letter are not significantly different at $P<0.05$.

	Disease characteristics					
	Latent period†		Lesion type‡		Sporulation§		
Genotype	Seedling	Adult-plant	Seedling	Adult-plant	Seedling	Adult-plant	Spore/ml
Jackson	5.7 C	9.8 D	LCL	ND	1.0 A	ND	ND
Stander	8.2 B	11.3 C	LCL	LCL	0.8 B	0.8 A	3.8 x 10^4 A
Osiris	- -	- -	NL	NL	0.0 C	0.1 C	0 C
UNA 80	10.5 A	13.7 B	DL	DL	0.9 AB	0.5 B	1.5 x 10^4 B
Zavila	11.2 A	15.0 A	DL	DL	0.9 AB	0.4 B	1.1 x 10^4 B

† Days from inoculation to formation of the first young lesion of scald.

± ND: No data; NL: No lesion; LCL: Large coalescing lesion; DL: Discrete lesion.

§ Seeding: The proportion of samples with sporulation out of 6-9 cases studied per each genotype. Spore/ml: The number of spores conidia washed from pieces of leaf tissues (10 mm) bearing a lesion into 2 ml of water were counted as the number of spores per lesion.

Table 5-2. Mean squares for the disease score and the arcsine transformed calcium, potassium, silicon, and sodium levels of the leaf samples as % of gold used as coating on the surface of leaf samples studied. (A) Nested two-way analysis of variance; (B) Factorial analysis of variance.

A

		Mean square				
S.o.V[†]	df	Score	K	Ca	Si	Na
Rep	2	0.07	0.36	1.91	33.72	3.42
Gen	4	14.11 **	88.00 **	52.17 **	117.85 **	89.79 **
Iso(Gen)	27	5.96 **	37.76 **	44.39 **	145.00 **	115.92 **
Error	62	0.17	6.86	1.96	16.40	245.31

B

		Mean square				
S.o.V	df	Score	K	Ca	Si	Na
Rep	2	0.07	0.2	0.7	56.02	6.28
Gen	3	11.96 **	108.5 **	13.7 **	48.74	223.1 **
Iso	3	23.24 **	142.9 **	66.5 **	218.60 **	113.9 **
Gen x Iso	9	1.67 **	30.1 **	37.7 **	59.22 *	148.4 **
Error	30	0.17	9.7	0.9	25.72	5.29

† S.o.V: Source of variation, Rep: Replicate, Gen: Genotype, Iso: Isolate.

*, ** Significant at $P<0.05$ and 0.01, respectively.

Table 5-3. Mean, standard deviation, and groupings of disease scores (0-4) for studied barley cultivars challenged with different isolates of *Rhynchosporium secalis*.

	Mean disease score ± Standard deviation and Grouping*				
Isolate\Genotype	Stander	Osiris	Zavila	UNA 80	Jackson
Iso 5	3.8± 0.2 a	0.3± 0.4 b	2.5± 0.2 c	3.3± 0.2 ab	-
Iso 7	-	-	2.7± 0.2 bc	3.8± 0.3 a	3.5± 0.3 a
Iso 11	3.7± 0.4 a	1.7± 0.5 a	3.3± 0.4 a	3.8± 0.2 a	3.7± 0.3 a
Iso 13	3.3± 0.4 a	0.3± 0.4 b	3.2± 0.2 ab	3.5± 0.3 a	3.5± 0.3 a
Iso 14	3.2± 0.2 a	-	0.3± 0.4 d	-	3.5± 0.3 a
Iso 18	3.8± 0.3 a	-	3.3± 0.3 a	0.5± 0.3 c	3.8± 0.2 a
Iso 19	-	-	3.7± 0.5 a	2.7± 0.5 b	3.8± 0.3 a
Not Inoculated	0.0± 0.0 b	0.0± 0.0 b	0.0± 0.0 d	0.0± 0.0 c	0.0± 0.0 b

* Means with the same letter within each barley cultivar are not significantly different according to Tukey's multiple mean test ($P < 0.05$).

Table 5-4. Mean/LS-means and groupings for disease scores (0-4) and the arcsine transformed calcium, potassium, silicon, and sodium levels of the leaf samples as % of gold used as coating on the surface of leaf samples studied for (A) the barley cultivars studied, and (B) the isolates inoculated on each cultivar.

A

	Mean, Grouping*				
		Element (as % of Au)			
Genotype	Score	K	Ca	Si	Na
Stander	3.0 a	10.1 b	13.0 a	11.1 a	4.8 d
Osiris	0.6 c	13.6 a	12.5 ab	3.6 c	9.4 a
Zavila	2.4 b	10.5 b	11.6 b	9.8 ab	6.2 c
UNA 80	2.5 b	8.0 c	9.9 c	7.7 b	10.1 a
Jackson	3.1 a	7.7 c	9.1 c	9.3 ab	7.7 b

B

		LS-Means (as % of Au) and Grouping*			
Genotype	Iso†	K	Ca	Si	Na
Stander	NI	7.15 bc	9.06 c	2.41 c	1.93 b
	5	6.45 cd	7.87 c	4.40 c	4.81 ab
	11	6.87 cd	9.23 c	8.84 b	4.09 ab
	13	9.85 bc	19.20 a	13.26 b	7.18 a
	14	11.28 b	19.92 a	28.86 a	5.13 ab
	18	19.18 a	12.52 b	8.72 b	5.66 a
Osiris	NI	13.06 a	11.64 b	0.06 b	11.83 a
	5	13.26 a	12.18 b	3.19 ab	9.11 ab
	11	13.58 a	10.00 b	3.53 ab	7.73 b
	13	14.67 a	15.97 a	7.56 a	8.91 ab
Zavila	NI	7.70 c	7.75 c	1.76 c	4.53 cd
	5	7.98 bc	9.80 c	4.11 c	5.32 bc
	7	11.52 abc	12.59 b	8.39 b	9.14 a
	11	11.04 abc	14.29 b	18.25 a	8.32 ab
	13	12.14 ab	8.66 c	7.95 bc	6.62 abc
	14	10.05 abc	9.97 c	3.07 c	1.61 d
	18	12.35 a	12.47 b	23.41 a	6.88 abc
	19	11.55 abc	17.50 a	11.35 b	6.82 abc
UNA 80	NI	1.23 b	4.43 d	0.05 c	15.22 b
	5	1.30 b	14.03 b	10.79 ab	32.72 a
	7	9.19 a	5.73 d	4.04 c	4.39 c
	11	10.44 a	10.73 c	3.76 c	3.99 c
	13	12.16 a	12.12 bc	15.13 a	5.29 c
	18	10.50 a	5.26 d	5.64 bc	5.16 c
	19	11.03 a	16.90 a	14.71 a	3.80 c
Jackson	NI	0.81 c	5.08 c	0.06 c	23.05 a
	7	7.19 b	8.98 ab	7.61 b	6.29 bc
	11	7.88 ab	10.92 a	19.62 a	7.49 b
	13	11.56 a	8.24 b	9.06 b	4.32 bc
	14	9.70 ab	9.79 ab	11.34 b	4.53 bc
	18	7.94 ab	10.94 a	11.29 b	5.18 bc
	19	8.91 ab	9.53 ab	6.10 bc	3.25 c

† NI: Not inoculated.

* Means/LS-Means with the same letter are not significantly different according Tukey's pairwise test at $P < 0.05$, respectively.

Table 5-5. Means and groupings for disease and the arcsine transformed calcium, potassium, silicon, and, sodium levels of the leaf samples as % of gold used as coating on the surface of leaf samples tested for (A) the barley cultivars studied, and (B) the isolates inoculated.

A

	Mean as % of Au, % of Stander, Grouping*							
Genotype	K		Ca		Si		Na	
Stander	9.9	100 b	11.3	100 b	7.2	100 a	4.5	100 c
Osiris	13.6	138 a	12.5	110 a	3.6	50 a	9.4	209 b
Zavila	9.7	98 b	10.1	89 c	8.0	111 a	6.2	138 c
UNA 80	6.3	63 c	10.3	91 c	7.4	103 a	14.3	318 a

B

Isolate[†]	Mean as % of Au, % of NI, Grouping*							
NI	7.3	100 c	8.2	100 c	1.1	100 c	8.4	100 b
Iso.5	7.2	99 c	11.0	133 b	5.6	525 b	13.0	155 a
Iso.11	10.5	144 b	11.1	135 b	8.6	803 ab	6.0	72 c
Iso.13	14.5	200 a	14.0	170 a	11.0	1025 a	7.0	84 bc

* Means in (A) and (B) followed by % of Stander and NI as the checks were used in this study to indicate differences between susceptible and resistant cultivar, and uninoculated and inoculated treatments, respectively. Means with the same letter are not significantly different according to Tukey's multiple mean test at $P < 0.05$.

† NI: Not inoculated.

Table 5-6. Pearson's coefficients of correlation between the elements studied and disease scores for the genotypes within the isolates Iso5, Iso11, and Iso13 (n = 24).

	Iso 5			
	Score	**K**	**Ca**	**Si**
K	-0.380			
Ca	0.164	0.477 *		
Si	0.357	-0.232	0.220	
Na	0.374	-0.392	0.372	0.627 **

	Iso 11			
	Score	**K**	**Ca**	**Si**
K	0.114			
Ca	0.502 *	0.493 *		
Si	0.457 *	0.074	0.521 **	
Na	-0.436 *	0.124	-0.323	-0.159

	Iso 13			
	Score	**K**	**Ca**	**Si**
K	0.604 **			
Ca	0.388	0.798 **		
Si	0.691 **	0.335	0.395	
Na	-0.344	-0.222	-0.272	-0.340

*, ** Significant at P<0.05 and 0.01, respectively.

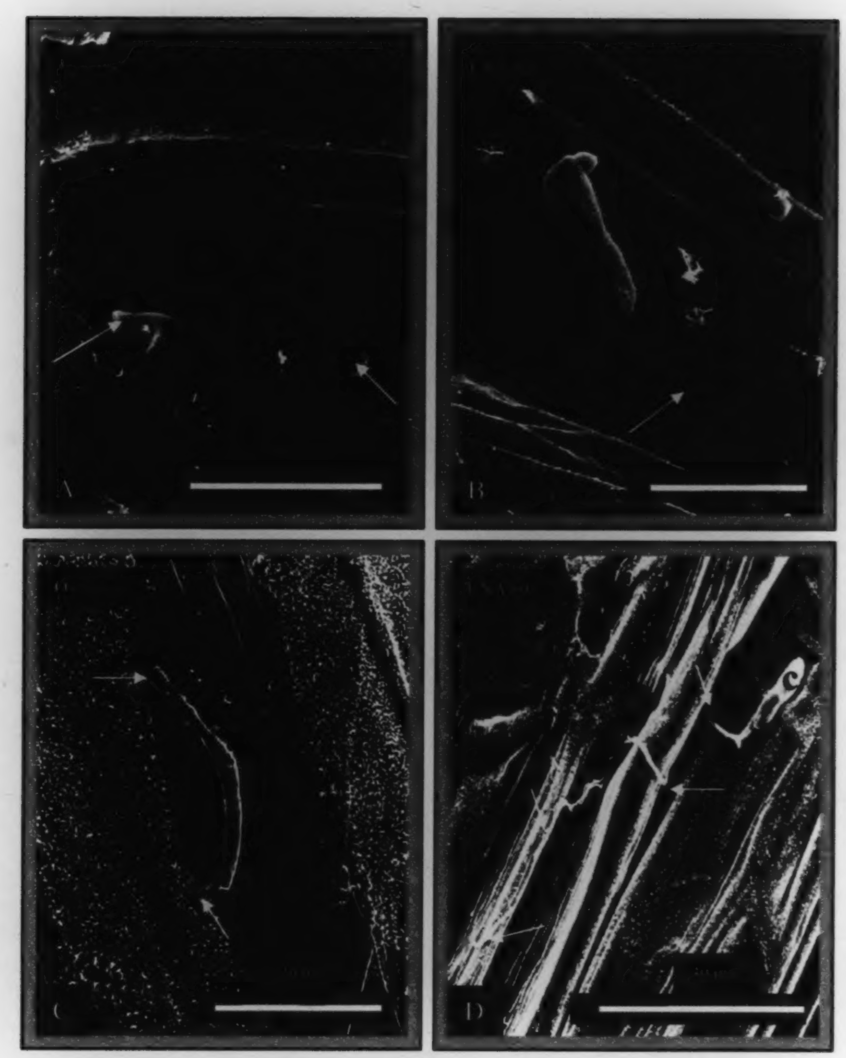

Figure 5-1. Scanning electron micrographs of barley leaf inoculated with *Rhynchosporium secalis*. (A) Conidium germination; arrows show the germination points; (B,C) Germ tube and appressorium formation; arrows point at the appressoria; and (D) Germination of a conidium on infected part of a leaf characterized by a very long germ tube with no appressorium; arrows indicate the germination.

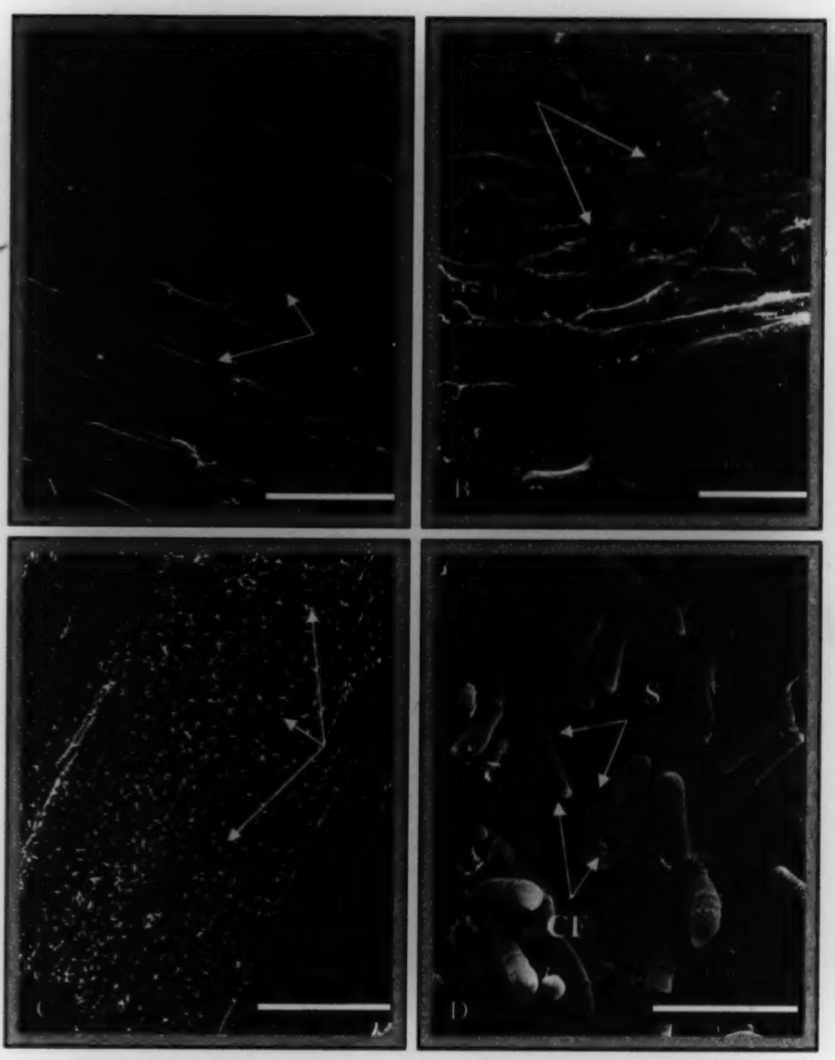

Figure 5-2. Scanning electron micrographs of barley leaf infected with *Rhynchosporium secalis*. (A) Subcuticular mycelia as indicated by arrows; (B) Initial conidia formation, arrows point to immature conidia; (C) Very susceptible interaction showing carpet sporulation all through the scanned area; and (D) Mature conidia of *R. secalis*, CF: Short conidiophores and S: Septa.

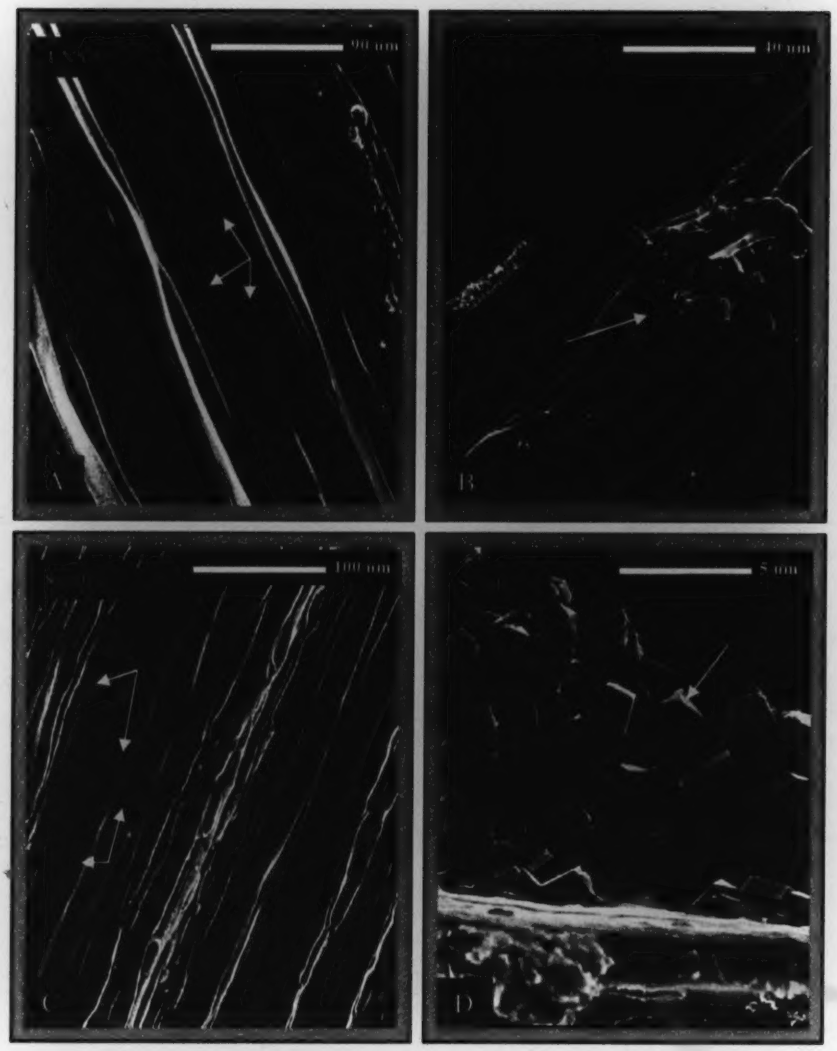

Figure 5-3. Scanning electron micrographs of barley leaf infected with *Rhynchosporium secalis*, where arrows show: (A) Crystals containing potassium beneath the epidermal cell wall; (B) Rod-shaped crystals containing calcium emerging through the surface of barley leaf; (C) Crystals beneath the epidermal cell wall containing calcium; and (D) Crystals containing calcium formed on the macerated epidermal cells.

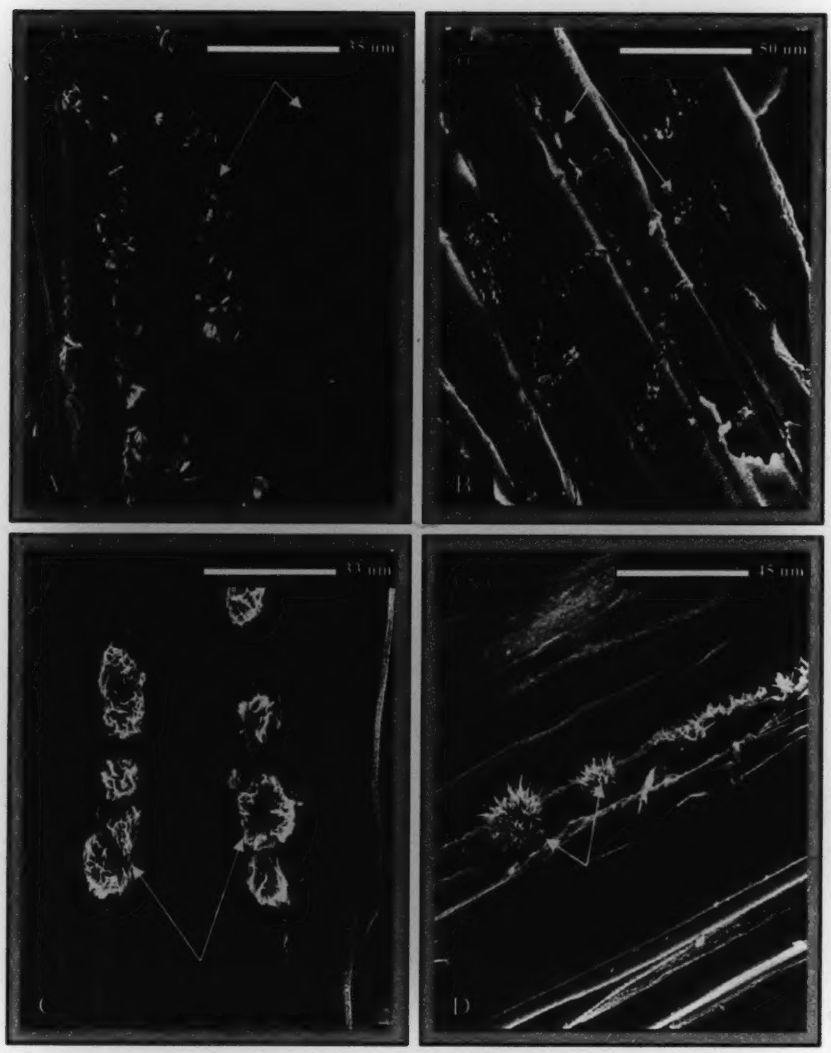

Figure 5-4. Scanning electron micrographs of crystals formed on the surface of barley leaves after inoculation with *R. secalis*, where arrows indicate: (A) Plate-like crystals and druses thereof containing potassium/calcium; (B) Bipyramidal crystals containing calcium/silicon; (C) Druses containing calcium/potassium; and (D) Raphide druses containing calcium. Note that all crystals were formed on top of the subcuticular mycelia as a result of after-inoculation response to the development of fungal hyphae in the plant cuticle.

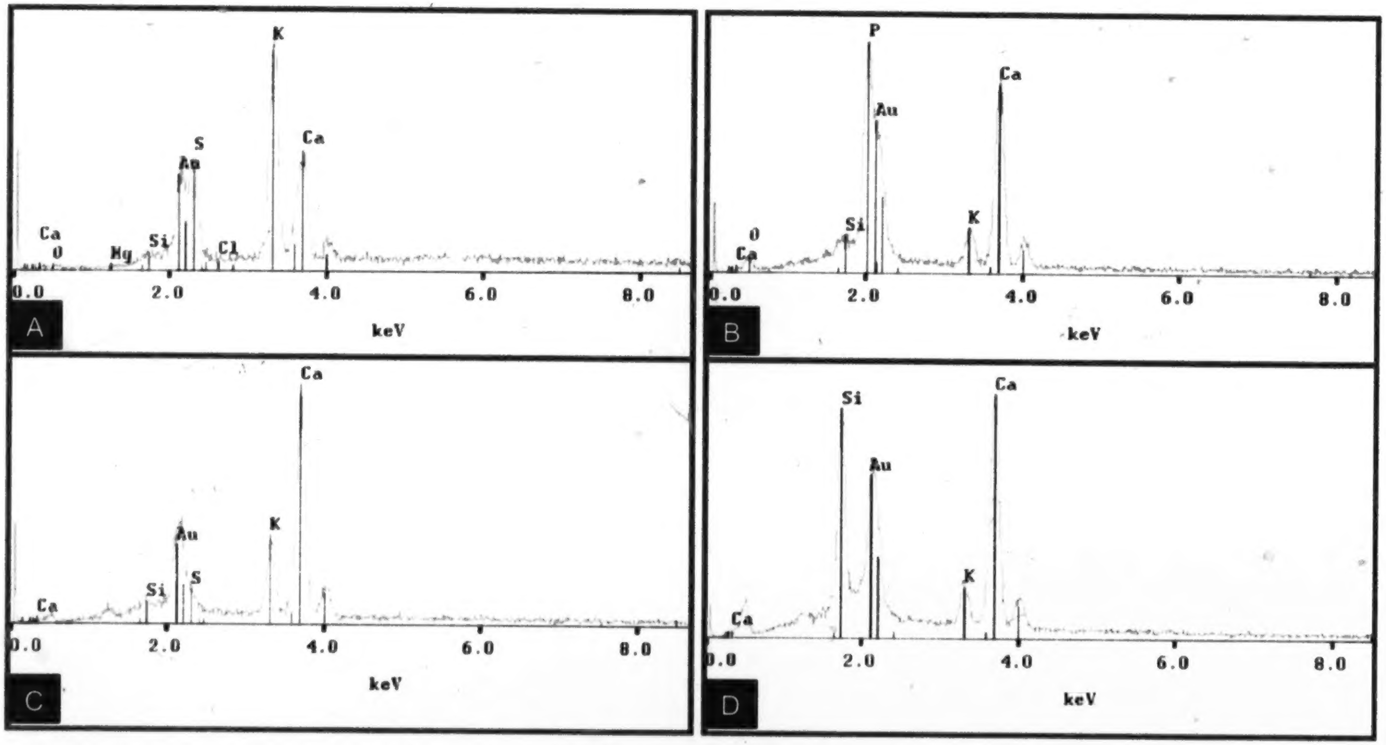

Figure 5-5. Energy-dispersive X-ray microanalysis spectra of crystals formed on the surface of barley leaves after inoculation with *R. secalis*. (A) Plate-like potassium/calcium containing crystals; (B) Raphide druses containing calcium; (C) Druses containing calcium/ potassium; and (D) Bipyramidal crystals containing calcium/silicon.

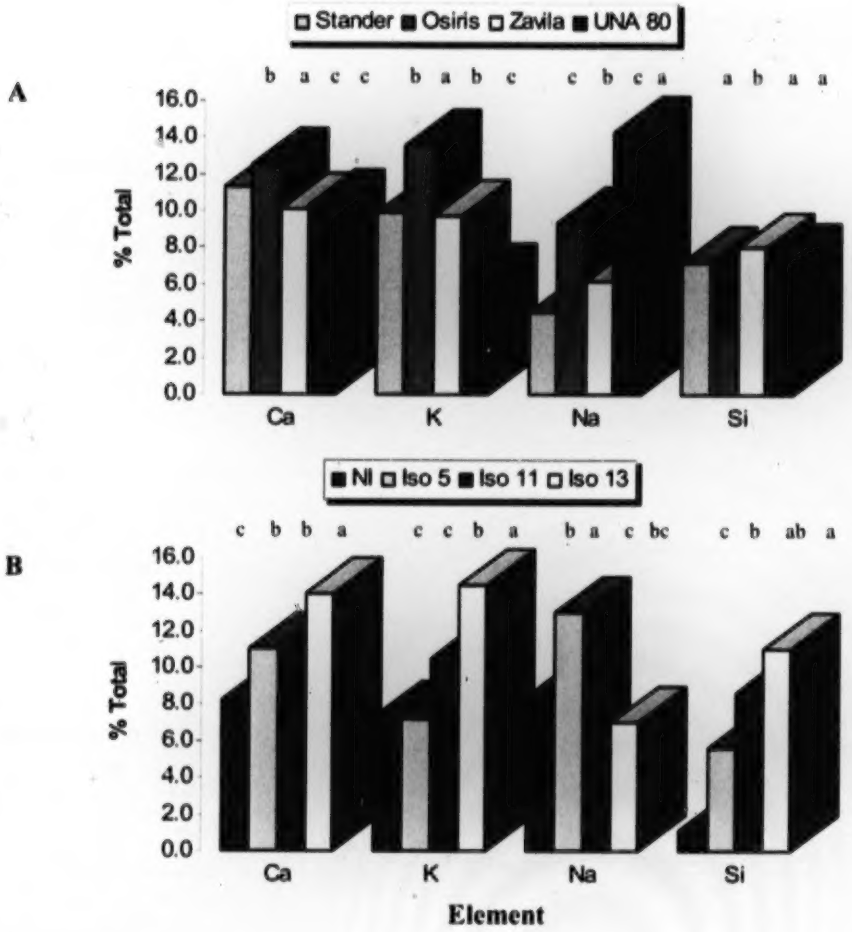

Figure 5-6. Bar graph representation of the differences among: (A) barley genotypes, and (B) scald isolates in terms of the barley epidermal cell's calcium (Ca), silicon (Si), potassium (K), and sodium (Na) measured using X-ray microanalysis. For each element, bars with different letter(s) are significantly different (Tukey's pairwise comparisons test at $p<0.05$).

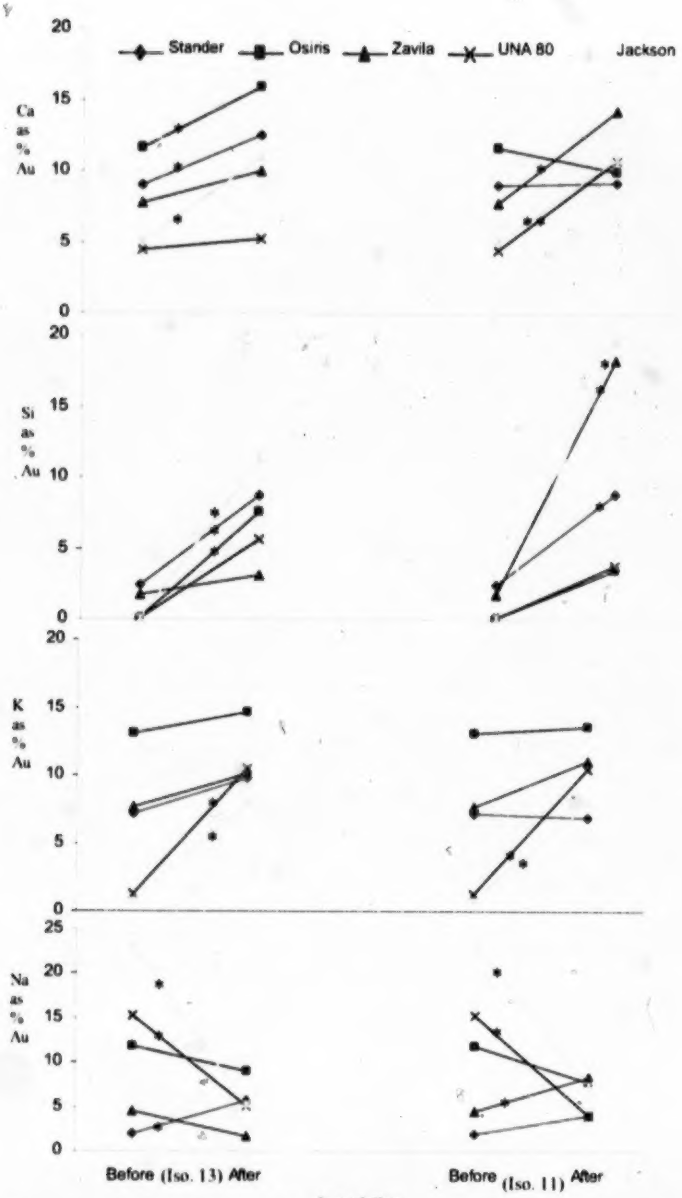

Figure 5-7. The trends showing changes in the barley epidermal cell's calcium (Ca), silicon (Si), potassium (K), and sodium (Na) as percentage of gold (%Au) used as coating on the surface of samples for the scanning electron microscopy and X-ray microanalysis, before and after inoculation.
* indicates significant changes.

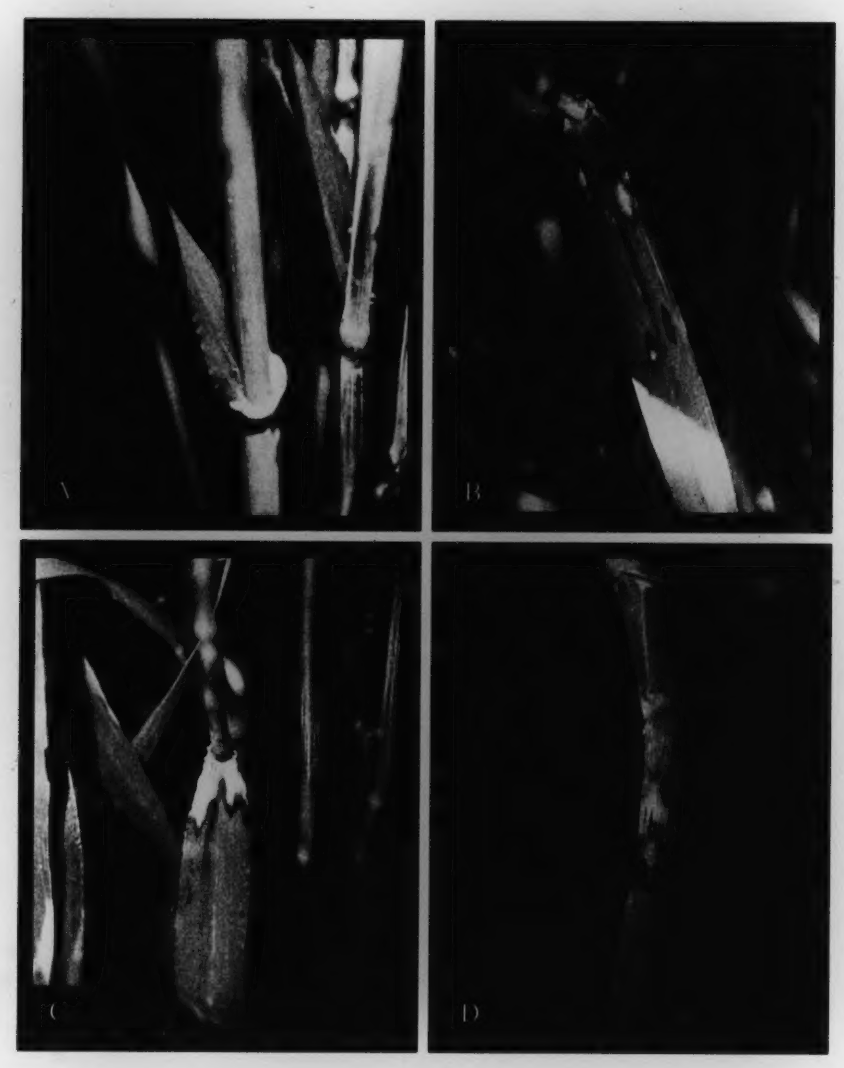

Figure 5-8. Leaf and sheath of the barley cultivar, Stander (susceptible to *Rhynchosporium secalis*). (A) Healthy leaf and sheath, (B) Infected leaf showing typical distinct lesions of scald at different stages of maturation, and a small infected area on the auricle (disease score 0-1 out of 4), (C) Heavily infected auricle (disease score 4 out 4), where the rest of leaf is not infected, and (D) Severe infection on sheath. Note in photograph A, the color of auricles in cv. Stander is chlorotic.

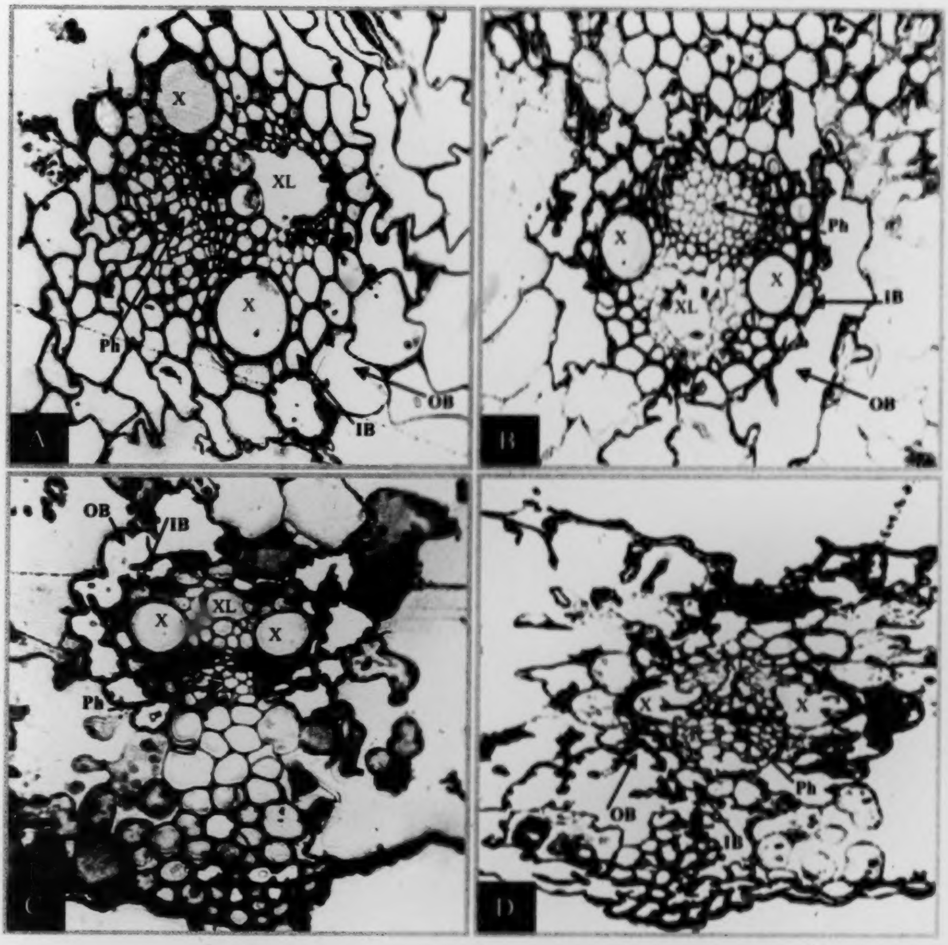

Figure 5-9. Light micrographs (transverse sections) of the barley leaf's auricle showing varying levels of damage caused by *Rhynchosporium secalis*. (A) Uninoculated leaf, (B) Damage in outer layer of the bundle sheath (disease score 3 out of 4), (C) Damage in inner layer of the bundle sheath (disease score 3 out of 4), and (D) Damage in xylem and phloem elements (disease score 4 out of 4). XL, X, P, IB, and OB indicate xylem lacuna, xylem, and phloem, inner and outer layer of vascular bundle sheath, respectively.

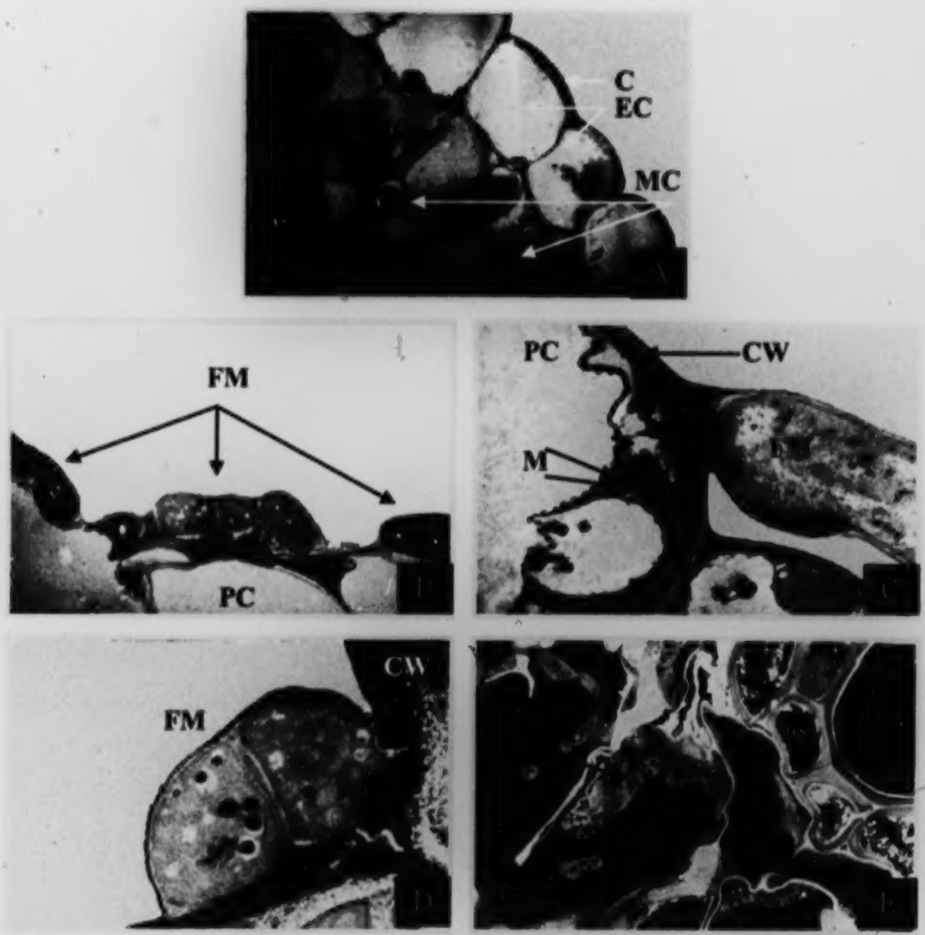

Figure 5-10. Transmission electron micrographs (transverse section) of the healthy and inoculated barley leaves showing compatible interaction with *Rhynchosporium secalis*. (A) Healthy epidermal and mesophyll cells, (B) Subcuticular hyphae in contact with the epidermis, (C) A mesophyll cell in direct contact with a fungal hypha, (D) Fungal hypha on a degrading plant cell wall in bundle sheath layer, and (E) The invading fungal mycelia surrounding vascular bundle sheath. Abbreviations: C: Cuticle; EC: Epidermal cell; MC: Mesophyll cell; IS: Intercellular space; FM: Fungal mycelium; PC: Plant cell; CW: Cell wall and BS, Bundle sheath.

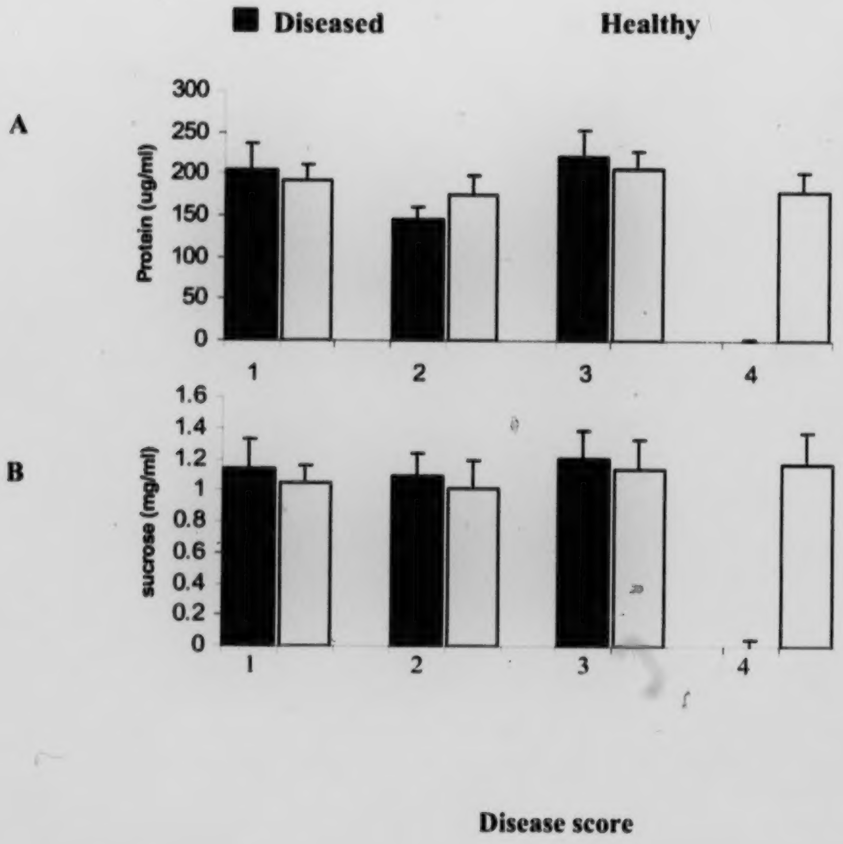

Figure 5-11. Bar graph representation of the differences among (A) protein, and (B) sugar contents of the vascular sap collected from diseased (blue bars) and healthy samples (yellow bars).

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Chapter 6

Genetics of slow-scalding resistance in spring barley[§]

6.1. Introduction

Barley (*Hordeum vulgare* L.) can suffer significant losses in yield and quality as a result of the scald disease caused by *Rhynchosporium secalis* (Oud.) J. J. Davis (Khan and Crosbie, 1988; Shipton *et al.*, 1974; Turkington *et al.*, 1998). The importance of the disease has increased in modern farming systems where zero or minimum tillage is continuously practiced (Bailey, 2002). Of different possible disease control measures, perhaps resistance breeding has been the most effective, and environmentally-friendly strategy. However, due to extremely high pathogenic variation of the barley scald pathogen, in many instances the fungus has been reported to have overcome the genetic resistance introduced into newly released barley cultivars (Xi *et al.*, 2003). Resistance to *R. secalis* can be governed by one or few major, and/or several genes with minor effects. Several different genes and QTLs have been identified to condition resistance to *R. secalis*, 14 of which have been considered as major genes (Chapter 1). Instability of major-gene resistance is of considerable concern to plant breeders and pathologists. Hence, slow-scalding resistance (S-SR) has been gaining increasing attention (Ginkel and Vivar, 1986; Helm *et al.*, 2001) as potentially it can play a crucial role in resistance breeding (Capettini *et al.*, 2002). Slow-scalding lines can exhibit a combination of compatible reactions to *R. secalis* at the seedling stage and adequate levels of resistance at the adult plant stage (Chapter 3). Their field resistance disease / progress during the course of epidemic can be characterized as being low to intermediate. Different sources of S-SR have been detected among the Canadian and ICARDA/CIMMYT scald resistant barley genotypes (Ginkel and Vivar, 1986; Sorkhilalehloo *et al.*, 2000; Sorkhilalehloo *et*

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al., 2001). Theoretically, the slow-scalding phenomenon can be classified as being partial resistance and can be recognized by a susceptible infection type coupled with a reduction in disease progress. However, the genetic basis and inheritance of slow-scalding is unknown and needs to be investigated.

This study was undertaken to investigate the genetic basis and inheritance of S-SR in three ICARDA/CIMMYT slow-scalding lines, and CDC Dolly, a Canadian slow-scalding variety.

6.2. Materials and Methods

To study the genetics of slow-scalding two parallel studies were conducted on different genetic populations derived from (1) three ICARDA/CIMMYT lines, and (2) CDC Dolly as donors of SSR. For the sake of simplicity, genetic populations used in these studies are named as (1) ICARDA/CIMMYT, and (2) CDC Dolly populations, respectively.

6.2.1. Genetic materials

Studies on ICARDA/CIMMYT populations

To obtain enough homogenous seed from the parental lines used in this study, a single designated seed of each parent was multiplied in the University of Alberta's indoor facility during the winter of 1999. Designated seeds of a set of three ICARDA/CIMMYT lines i.e. UNA 80, Zavila, and CI1240/Foma// CI16239.15D, and a susceptible cultivar, Stander (a common North American spring malting barley), all of which were six-rowed and spring types were planted under field conditions in a crossing plot in Edmonton, Canada during 1999. To obtain F1 seeds, all possible crosses were made between pairs of these genotypes. F1's were raised to F2 segregating generations in Edmonton during the summer of 2000. F2-drived F3 lines were obtained from F2 seeds under the University of Alberta's indoor adult-plant conditions (Chapter 2) during the winter of 2001. F4 seeds were harvested from F3 plants at the Edmonton Field Research Station during the summer of 2001. F4 lines of each cross were then advanced to F5 recombinant inbred lines (RILs) in Ciudad Obregon, Mexico during the winter of 2002. Single-seed descent methodology (SSD) was the basis of our methodology to advance each generation.

However, in order to have enough seeds for all locations/generation trials more than one seed from each spike was randomly collected to be raised to the next generation. The parents, F1's (15 plants for each cross), F2's (72 to 266 line from each cross), F3's (72 to 122 lines from each cross), and F5's (88 to 118 lines from each cross) were used to study the genetics of SSR in separate studies.

Studies on the populations of CDC Dolly

Two populations of CDC Dolly as a Canadian slow-scalder provided by Dr. B.G Rossnagel were investigated. All parental lines were spring types and two-rowed. The first population (DH) consisted of 200 SSD-RILs obtained from a cross between the designated cultivars CDC Dolly 6 and RFLP Harrington. The second population (DT) included 219 anther-derived doubled haploid RILs from a F1 between CDC Dolly and TR251. Harrington and TR251 were used as the susceptible parents.

6.2.2. Field locations and method of planting field plots

Field evaluations were carried out in three different locations, Edmonton (53°/33'N latitude, 113°/28'W longitude and 668 m elevation) and Lacombe (52°/28'N latitude, 113°/44'W longitude and 853 m elevation), Canada, and Toluca (19°/24'N latitude, 99°/12'W longitude and 2640 m elevation), Mexico. The environmental conditions at these locations are usually ideal for scald development and do not require any modifications. However, over-head irrigation was applied in Edmonton to reduce the temperature during hot days and to increase the humidity during dry periods. Ciudad Obregon in the Northwest of Mexico situated at 22°N latitude, 7m elevation was also used to advance ICARDA/CIMMYT F4 populations were advanced during the cold seasons at the aforesaid locations. This facilitated advancement of the segregating populations at the rate of two generations a year.

The plant materials for ICARDA/CIMMYT studies were space-seeded (5-10 cm apart) in rows 2 m long, whereas the CDC Dolly derived genetic populations (8-10 seeds) were hand-sown in hill plots at approximately 50 cm spacing. The depth of seeding was approximately 0.5 cm.

6.2.3. Inoculation

After the tillering stage (ZGS 31), mixtures of different isolates of *R. secalis* were applied among experimental plots as artificial inocula to support epidemics at all locations/years. Susceptible spreader rows were grown all around each of the replicates of experimental tests to enhance the rate and amount of infection during the course of epidemics. In Edmonton and Lacombe, a final concentration of 10^5 conidia/ml of six Canadian scald isolates, Iso.1, Iso.2, Iso.3, Iso.4, Iso.5, and Iso.6, belonging to Dr. J.P. Tewari's barley scald culture collection were used to inoculate the plants (Chapter 4). In Toluca, freshly cut infested barley shoots were placed in close contact with the experimental barley lines. The infested tissues were used as sources of inoculum in Toluca. This source of inoculum was all belonging to 10 different fully susceptible lines inoculated with a mixture of various isolates of scald collected from the previous season. More details about the inoculation techniques were given in Chapter 2 and 3.

6.2.4. Experimental design and traits

Studies on ICARDA/CIMMYT populations

During the summer of 2000, 15 F1 plants of each cross together with their respective parents were evaluated for their reactions to *R. secalis* in a randomized complete block design (RCBD) with three replicates under field conditions at Edmonton. The F2 and F3 generations of all crosses and their corresponding parents were then evaluated under similar conditions (RCBD with three replicates) in Edmonton, Lacombe, and Toluca during the summer of 2001. Similarly, scald severity was also assessed in the F5 recombinant inbred lines (RILs) of all crosses and their parents in Edmonton, during the summer of 2002. In all trials, disease severity for each entry was recorded using a visual assessment score of 0-9 (discussed in Chapter 2) three times before the physiological maturity at Zadoks growth stage (ZGS) of 85 (Zadoks *et al.*, 1974). Scald progress was then studied using area under the disease progress curve, which was calculated for the disease score and standardized based on the reaction of the most susceptible entries (SAUDPC, 1-100).

Studies on CDC Dolly populations (DH and DT)

In the 2000 and 2001 field experiments, DH was seeded in hill plots and studied through an alpha lattice design with 4 and 3 replications in Edmonton and Lacombe, respectively. The other population, DT was assessed as a back-up study with 3 replicates in Edmonton and only one replicate in Lacombe during 2000 and 2001. A complete set of each of these populations was also studied as an observation nursery at Toluca, CIMMYT during the summer of 2001. The RILs, DH lines, and their parental genotypes were evaluated for their percentage leaf area scalded (LAS as %severity) three times before physiological maturity. The last %LAS was considered as final disease severity (FS). Standardized area under the disease progress curves (SAUDPC) was then calculated for %LAS (AS, 0-100). Terminal severity (TS, 0-9) was also recorded as plants final disease score at ZGS 85.

6.2.5. Statistical analyses

Statistical analyses were performed using the Proc GLM and Proc Corr (SAS Institute Inc., 1989) and diallel analysis softwares DIALLEL (Burow and Coors, 1994), and DIAL (Ukai, 1989).

Studies on ICARDA/CIMMYT populations

Correlation analysis was used to study the relationships between the traits as well as the association between different generation trials within test locations. Hence, Spearman's coefficients of correlation were calculated for rankings of both parental lines and their respective crosses mean TS and AUDPC values.

Analyses of variance for all diallel trials were performed using the SAS program. The difference between the mean SAUDPC of a cross progeny and the corresponding mean of the parents (mid-parent value) was considered as heterosis for slow-scalding. Using the Tukey's minimum significant difference values (HSD), the parental mean ± HSD for SAUDPC were used to detect lines with transgressive segregation in each cross. In order to study the general combining ability (GCA), specific combining ability (SCA), and reciprocal effects (RE) of the parents, the total variances of F1, F2, F3, and F5 data were partitioned to their components i.e. σ^2_{gca}, σ^2_{sca}, and σ^2_{r}, respectively, according to Hayman's and Griffing's diallel analyses (Baker, 1978; Griffing, 1956; Hayman, 1954a;

b; Mather and Jinks, 1974). Genotype effect was considered to be fixed in all diallel analyses. In Griffing's diallel analysis, methods I and III were compared to determine the presence/absence of parental effects. Baker ratio, $2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca})$, was used to compare the contribution of additive and non-additive effects of genes in S-SR. The contributions of GCA, SCA, and RE were also studied relative to each other. Using the DIAL program, the additive (D) and non-additive (H1 and H2) effects were estimated for the F1 and F2 data according to Mather and Jinks (1974). Additive (Add) and non-additive (Dom) effects were also calculated using the Diallel program according to Griffing (1956a).To compare the relative importance of each of these components of genetic variance, a ratio was calculated based on the proportion of each effect relative to the total variance. Further, the genetics of S-SR was studied through estimation of heritability for the F1, F2 , and F5 generations using the genotypic components of variance according to Poehlman and Sleper (1995). The array variances (V_r) and covariances (W_r) of F1 families were also calculated and plotted according to Hayman (1954a) to obtain more genetic information about the non-additive effects of the genes involved in S-SR. The number of segregating factors (gene number) was finally determined using the Wright, and Bjarko and Lines gene number formulae GNF1-3 (Bjarko and Line, 1988; Wright, 1968).

Studies on the populations of CDC Dolly

Separate and combined analyses of variance were performed for the traits studied using Proc MIXED of the SAS program. Parental mean ± standard deviation for AS was used to detect lines with transgressive segregations in each population. The genetics of slow-scalding was studied through estimation of the number of genes (Bjarko and Line, 1988), and the heritability of S-SR using the variance components of genotypic values (Poehlman and Sleper, 1995).

6.3. Results

6.3.1. Studies on ICARDA/CIMMYT populations

Scald severity was high and differentiating in all locations/years as indicated by the means for TS and SAUDPC values of the susceptible parental lines (Table 6-1). The susceptible parent, Stander, showed a high degree of susceptibility across all trials (TS = 6.2-8.7; SAUDPC = 80.7-93.1) whereas the slow-scalding lines demonstrated low to intermediate quantitative reactions to the applied inocula of *R. secalis* (TS = 0.2- 4.8; SAUDPC = 0.7-53.2). Differences were observed among resistant parents in different generation trials. Among parental slow-scalders, Zavila exhibited higher resistance levels in Canada while there were no significant differences among resistant parents in Mexico. On the other hand, although UNA 80 showed the highest values of SAUDPC in Canada, it had the lowest levels of scald severities among slow-scalding parents in Toluca, Mexico. The level of resistance in Zavila decreased from its highest level close to immunity at F1 generation in the year 2000 (TS = 0.7 ± 0.6) to its lowest level during the summer of 2000 (TS = 2.4 ± 1.1) when the F5 generation was under study. The latter was still below the level of its most compatible type of reaction for Zavila at CIMMYT (TS = 4.3 ± 0.9). Within individual crosses none of the parental lines exhibited immunity at field conditions, but some lines from RxR intercrosses were completely resistant to scald and showed no symptoms of the scald disease.

The mean values of SAUDPC or TS representing the scald severities of the parents and their crosses varied slightly among the generation trials. However, as shown in Table 6-2, the correlation analysis indicated that within each location there were significant associations among the generations for each cross as revealed by significant Spearman's coefficients of correlation for the rankings of both parental lines and their respective crosses mean disease severity values (r = 0.84-0.97, P< 0.01). The high correlation between TS and AUDPC values (the diagonal bolded numbers) indicated that either trait could result in similar classification of the studied lines/parents (r = 0.87-0.99, P< 0.01). However, we used the former trait (SAUDPC) to study the genetics of slow-scalding as it could represent the disease progress in a more efficient fashion (Campbell and Madden, 1990; Kranz, 1988).

6.3.2. Deviations from mid-parents

Table 6-1 shows the values of SAUDPC for all crosses including reciprocals. Mid-parent values (MP), deviation from mid-parents (DMP), skewness (SKW), and standard error (SE) of SAUDPC for all generation trials are given in Table 6-3. As in other traits, heterosis (hybrid vigor) for slow-scalding was defined as the increase in resistance level of a hybrid progeny in relation to the average of the parents, referred to as mid-parent value according to Poehlman and Sleper (1995). Only negative DMP values could indicate heterosis for slow-scalding resistance. In all crosses, F1 plants demonstrated heterosis for SAUDPC. In addition to that of F1's, the negative values of DMP were also observed in F2, F3, and F5 generations of most crosses where the absolute values of deviation from mid-parents were higher than those of the respective standard error in each generation. As shown in Figures 6-1 and 6-2, no specific pattern could be identified for describing changes in the mean of population from one generation to another. Figures 6-1 and 6-2 also show the frequency distribution of SAUDPC of F2 plants of all studied crosses and the population means for different generations/locations coupled with their corresponding parental severities as indicated by arrows. The F2 and F3 population means of all crosses for Edmonton, Lacombe and Toluca, and F1 and F5 data for Edmonton are shown in these tables. In summary, the populations of all susceptible x resistance crosses and most resistance intercrosses had a mean severity within the range of average SAUDPC for their parents skewed toward the more resistant parent. In some instances, the crosses UxC and ZxC were exceptions, where their population means exhibited disease severity even lower than those of more resistant parents.

6.3.3. Frequency distribution and transgressive segregation

Frequency distributions of barley lines in the populations investigated in this study were continuous. Also, both resistance and susceptible transgressive segregations from the parental values were seen among the populations studied. Tables 6-4 to 6-5 represent these features for the populations of susceptible x resistant crosses (SxR) and resistance intercrosses (RxR), respectively. The frequency distributions of the SAUDPC of all F2, F3, and F5 lines were continuous. Additionally, transgressive segregation was observed among both SxR and RxR crosses toward the lowest (resistance) and highest (susceptible) SAUDPC values.

6.3.4. Diallel analyses

Complete F1, F2, F3, and F5 diallel crosses were used to investigate the inheritance of slow-scalding resistance in ICARDA/CIMMYT parental lines. Mean squares of SAUDPC of all different generation trials for both Hayman's (1954) and Griffing's (1956) diallel methods are given in Table 6-6. Significant differences existed among genotypes, crosses, and parents in all generations for the levels of scald progress as determined by the values of SAUDPC. As shown in Table 6-1, scald progress in Stander, as the susceptible parent of all SxR crosses, was significantly higher than those of the slow-scalding parental lines and means of their corresponding crosses in all generation trials. However, differences among the slow-scalding parents and means of their respective crosses were not significant in all circumstances. Additionally, there were significant differences among crosses in terms of their mean SAUDPC in all generation trials. The average disease progress in a given resistant x susceptible cross was significantly different from that of corresponding resistant parent intercrosses which had the same slow-scalding parent in its pedigree. For example, the average SAUDPC for the crosses of Stander and Zavila were significantly higher than those of Zavila x UNA 80 or Zavila x CI1240/Foma//CI16239.15.D (Table 6-1). Except for F2 populations at Edmonton during 2001, parent versus cross (P vs. C) comparisons were significant in all generation trials. The significance of P vs. C was an indication of the presence of non-additive genes in the trait studied as shown by Mather and Jinks (1974). On the other hand, the diallel analyses confirmed the significance of general combining ability effects (GCA in Griffing's and component "a" in Hayman's methods) in all cases, indicating the presence of a significant additive effect of gene(s) governing S-SR. Specific combining ability was also significant in most studied generations/diallel methods. The significant mean square values of this part of total variance could be an indication of the presence of non-additive effects including dominance and/or epistasis. Although both GCA and SCA showed significant mean square values, the SCA effects were smaller than GCA effects in all instances. The reciprocal effects, components "c" and "d" in Hayman's, and "RE" in Griffing's diallel analyses, were only statistically significant in some generation trials; and, their mean squares were much smaller than the combining abilities components. The reciprocal values of all crosses are also presented in Table 6-1. Except for those cases indicated by asterisks as reciprocal crosses having different scald progress rates, there were no significant differences among all reciprocal pairs of other crosses. Our observations indicated that excluding the slow-scalding line, no parental effects could be assigned to the rest of parents. In most studied generations in Edmonton when used as the maternal parent, CI1240/Foma//CI16239.15D contributed to lower values of SAUDPC. This might indicate to breeders that this line may not be a useful recurrent parent. Nevertheless, this reciprocal effect was not found at other locations.

Similarities and differences were found between different models and methods used to study the parents and their crosses through diallel analysis. For example, the values of SCA in Griffing's methods I and III were equal to the components "b" (non-additive) and "b3" (part of dominance deviation that is unique to the corresponding F population) of Hayman, respectively. Different analyses also resulted in various mean squares for SAUDPC of each generation trial. However, similar conclusions could be drawn for the same data analyzed using different methods. For example, the degrees of freedom (df) and the significance of mean squares of GCA in Method I and III in Griffing's diallel analysis were similar. In contrast, SCA in these methods had different df's but similar significance levels for all the generations and locations except for the generation F3 in Toluca where the SCA in Method III was not significant. For reciprocal effects estimated by the different methods applied, similar results were found but Hayman's model provided more details as to whether the reciprocal effects were due to the contributions of maternal component (c) or not.

The estimates of GCA and SCA for SAUDPC are given in Table 6-7. Only negative GCA/SCA values showed contribution towards more scald resistance. Hence, genotypes and crosses with the lowest GCA and SCA values were identified as the best general combiners and specific crosses for slow-scalding resistance, respectively. In Canada, Zavila was the best general combiner across all different locations/years whereas in Toluca, CI1240/Foma//CI16239.15D showed the most negative estimates of GCA. Stander had the largest positive GCA for SAUDPC through all test generations. Among the SxR crosses, Zavila and CI1240/Foma//CI16239.15D showed the best SCA with Stander in Canada and Mexico, respectively. In contrast, the cross of UNA 80 and CI1240/Foma//CI16239.15D was considered the best specific cross with the greatest average heterosis across all generation trials.

Table 6-8 presents estimates of the genetic components of S-SR, their contributions to the total genetic variance, and narrow-sense heritability of the SAUDPC in different generation trials studied using different diallel analyses methods. The estimates of GCA (σ^2_{gca}), SCA (σ^2_{sca}), RE (σ^2_r), additive (D/Add) and dominance (H1, H2/Dom), and also the contribution of each effect to total variance in respective generation from analyses which performed with and without parents were given in this table. "Contribution" showed the proportion of each effect relative to total variance whereas Baker's components of variance ratio represented $2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca})$. The closer these ratios were to unity, the greater relative importance was in explaining the total variance. It was demonstrated that GCA effects explained a significant portion of the total variance in the analyses (0.77 - 0.97) whereas the reciprocal effect showed the least amount of ratios among the components of genetic variance (0.002-0.003). Baker's ratios for the trait studied in different generations were all indicative of a significant contribution of additive effect in the inheritance of S-SR. Additive/dominance ratios (D/H1; Add/Dom) also showed that the additive effect was the major component of genetic variance of each generation. However, there were discrepancies between the results obtained from analyses with and without parents. Additive/dominance ratio was found to be greater in the same generation trials where the effects of parents were excluded from the analysis. Accordingly, the estimates of heritability calculated for a given generation trial was changed upon excluding the parents from the analyses. The heritability of the SAUDPC for all generations was generally quite high (0.80-0.95 in analyses without parents. However, the generation trials in Canada showed higher heritabilities than those of generation trials studied in Mexico.

Regression of array covariance (W_r) on array variance (V_r) for SAUDPC of F1 families used to assess genetic control of scald in ICARDA/CIMMYT populations is given in Figure 6-3. Note that red line represents the slope of unity where dominance (H1) and additive (D) gene effects are theoretically considered to be equal. The dotted blue line shows indicates where frequency of dominant (p) and recessive (q) alleles/genes are equal whereas the frequency of p is increased for parents with lower W_r values. The V_r-W_r graphical analysis revealed that of the slow-scalding parents, Zavila and

CI1240/Foma// CI16239.15D showed similar gene actions and distribution whereas a combination of additive and dominant effects coupled with a higher frequency of recessive alleles were shown for UNA 80. The deviation of the slope of the V_r-W_r line from unity was significant (t = 6.23; P = 0.0248). Hence, in addition to dominance and addictiveness, epistasis might be considered as one of the gene actions involved in S-SR.

The results also revealed that S-SR in the slow-scalding parents was controlled by one to three genes with additive effects (Table 6-9). The application of different gene formulae corresponding to different generations resulted in similar number of genes for a given cross. However, these estimates varied across different locations. For example, the number of genes estimated for the cross, UNA 80 x Stander conditioning resistance in Edmonton, Lacombe and Toluca were approximately 3, 1, and 3, respectively. In general, the gene numbers estimated in Lacombe were found to be less than those in Edmonton and Toluca.

6.3.5. Studies on the populations of CDC Dolly

Mean squares for final disease severity (FS), standardized area under the disease progress curve (AS), and terminal severity (TS) of the populations DH and DT are given in Table 6-10. Significant differences were observed among the genotypes (parents and RILs) of both populations for all the traits studied in different locations/years. According to a Bartlett's test (Steel *et al.*, 1997), significant differences were observed among the error variances of the traits studied except for those assessed in Edmonton for the population of DH. Table 6-11 shows the combined analyses of variance which were only performed on the FS, AS, and TS data of the population DH studied in Edmonton during the summers of 2001 and 2002. Disease levels were statistically different between the test environments, as were genotype, and genotype x environment interactions for all traits.

Figures 6-4 and 6-5 show the frequency distributions of AS for scald severity of 200 recombinant inbred lines of the DH population, and 219 double haploid recombinant inbred lines of the population DT, respectively, studied in Edmonton, Lacombe, and Toluca. The CDC populations had continuous quantitative distributions for the traits studied in all locations. Additionally, RILs with transgressive segregations were observed for both test populations in all test locations/years. Table 6-12 shows the number of transgressive segregants observed for the populations studied. Lines exhibiting lower (mean-std) and higher (mean+std) disease severity than those of the respective parents were considered as resistant (R), and susceptible (S) transgressive segregants, respectively.

Table 6-12 also shows the means and standard deviations of the parents for the populations of DH and DT. CDC Dolly showed a compatible reaction with *R. secalis* characterized by low to intermediate levels of disease severity under favorable conditions for the scald pathogen epidemics across the test environments in Canada. Hence, CDC Dolly reacted as a slow-scalding line only in Canada whereas in Mexico, its field reaction to the applied spectrum of scald isolates was highly susceptible. Significant differences in disease progress were observed between the susceptible cvs. Harrington and TR 251 Stander as the susceptible parents and the slow-scalding parent, CDC Dolly (Figure 6-6). Among the RILs, there were many instances with disease progress being lower, similar, or even greater than those of parental lines. The results obtained from Toluca showed that a portion of the resistance mechanism in CDC Dolly was race-specific. However, the existence of RILs with field resistance demonstrated that non-races specific genes with minor and additive effect contributed to S-SR.

Disease progress of a set of three RILs for each of the DH and DT populations together with their corresponding parents assessed in Edmonton, Lacombe, and Toluca are illustrated in Figure 6-6. In the DH population, RILs such as DH110, DH99, and DH96 were consistently resistant, intermediate, and susceptible over all test environments. Similar results were obtained with respect to DT89, DT205, and DT72 in the DT population. Regardless of stable reactions of the aforesaid RILs, there were several instances that showed RILs resistant in one environment and susceptible in another or *vice versa*. Additionally, as shown in these graphs, disease progress in both test populations and over all test environments were differentiated. The artificial inoculations created relatively high epidemics that easily differentiated genotypes with different levels of resistance. However, disease severity in Edmonton seemed to be less than that observed in Lacombe. Toluca also showed a greater level of the scald disease as compared to levels observed in Canada. The differences among the population means of a given cross also reflected variability among the test locations. Mean and standard deviation for the populations of DH and DT are presented in Table 6-12.

The genetics of slow-scalding was further studied through estimation of the gene number and the heritability of resistance genes by analyzing the variance components of phenotypic values (Table 6-13). The narrow-sense heritability estimated for the traits studied in both the DH and DT populations were very high in all locations ranging from 0.78-0.92. This study also revealed that depending on the test population and the traits studied, 3 to 5 minor genes with additive actions were found to be involved in S-SR. Using the combined data for the DH population, approximately, 3, 4, and 4 genes were found for the traits FS, AS, and TS, respectively, whereas 4 genes were considered to condition S-SR in the DH population studied in Lacombe. For the DT population, on the other hand, 4, 5, and 3 genes were estimated for FS, AS, and TS, respectively.

6.4. Discussion

Similar to slow-rusting in wheat, the expression of slow-scalding would vary in different epidemics; and various factors such as the pathogen race, location, and season of testing may contribute to the variance of this type of resistance (Van der Plank, 1984). It was observed for both studies on ICARDA/CIMMYT and CDC Dolly populations that the slow-scalding of a genotype was not equally effective in different generations/locations/years studied. These types of discrepancies/variations could be explained by different reactions of plant genotypes to various environmental conditions and also to their interactions with local/natural inocula of the disease pathogen at different years/locations where a given generation was tested (Andres and Wilcoxson, 1986). A genotype x environment interaction is dependent on the influence of disease including the pathogenicity of existing isolate populations, intensity of epidemics, interactions of the pathogen with the environment or the plant, or even second degree interaction of plant x pathogen x environment.

It was shown than the reaction of a given slow-scalding line may or may not be stable over different locations/years. This indicated that slow-scalding could also be under the control of race-specific type of resistance. Race-specific resistance is usually considered synonymous to an unstable resistance conditioned by major genes (Agrios, 1997). As some major genes may confer horizontal resistance (Cselenyi *et al.*, 1998) and minor-genes might contribute to vertical resistance components (Parlevliet, 2002; Scott *et al.*, 2000), it is better not to treat major-gene resistance as effectively synonymous with vertical resistance and minor-gene resistance with horizontal resistance. Additionally, despite the fact that major-gene resistance is usually considered to be unstable whereas minor-gene resistance is called durable, there are instances such as the one in the wheat-rust pathosystem that confirm that the major-gene resistance may last longer and benefit breeders as a durable type of resistance (McIntosh *et al.*, 2001). With respect to the potential of slow-scalding lines for providing a more stable resistance over various years/locations and also considering the fact that they carry genes with additive effects and high heritabilities, it is suggested that RILs can be used as sources of durable resistance to scald. Interestingly, some RILs of the populations of CDC Dolly in Toluca, where even the slow-scalding parent were found to be fully susceptible, reacted as resistant. This indicated that in addition resistance attributed to a major gene(s), quantitative/non-race specific background resistance is also likely to condition S-SR in CDC Dolly.

In all SxR crosses and most RxR intercrosses of ICARDA/CIMMYT F1 families, at least some plants demonstrated a severity lower than that of the mid-parent values for SAUDPC, with population means shifting toward the more resistant slow-scalding parents. This was an indication of contribution towards incomplete dominance. For those instances where the population mean showed a severity even lower than that of both parents, it was suggested that the additive effect and/or additive by additive non-allelic interaction (epistasis) could be playing a role in slow-scalding resistance. There is a belief that non-allelic gene interaction rather than overdominance may be responsible for resistant transgressive segregation (Jinks, 1956). Significant deviations from the mid-parental value have been used by some authors to indicate epistasis (Choo *et al.*, 1986; Robinson, 1999). In the present study, the results from the regression of array covariance on array variance suggested that non-allelic interactions were likely involved in the inheritance of S-SR. The presence of heterosis in the F2 populations, and the most SxR crosses in F3 and F5 generations where dominance variance were minimized due to shifting towards homozygosity, was another indication of significant contribution of genes with additive effects in slow-scalding parents.

Continuous distributions of lines in the present study indicates that slow-scalding resistance may have a quantitative inheritance. The continuum of frequency distribution of data has also been observed for qualitative traits with low levels of inheritance (Kuhn *et al.*, 1980; Lee and Shaner, 1985). However, as estimated in this study, the heritability of genes conditioning slow-scalding resistance was very high indicating that the genetic structure of slow-scalding in ICARDA/CIMMYT and CDC Dolly populations was most likely of quantitative nature, albeit that 3-4 genes were involved. We measured slow-scalding as disease development in the field. However, it may include the effects of several components of resistance such as latent period, spore production per lesion, size of lesions, and number of lesions with similar quantitative inheritance.

For the ICARDA/CIMMYT population, transgressive segregation was observed among both SxR and RxR crosses towards the highest and lowest SAUDPC values, implying the presence of different genes for resistance in the parents. The presence of transgressive segregation towards resistance in SxR crosses could imply that even the susceptible parents, Stander contained at least one minor gene for slow-scalding resistance. The combinations of genes in slow-scalding parents with the gene(s) in Stander have conferred some RILs significantly better scald resistance than that of the respective resistant parents. Similar results were obtained for the susceptible parents Harrington and TR 251 in CDC Dolly's populations, with similar conclusions. The other possibility to rationalize the contribution of Stander, Harrington, and TR 251 towards resistance is to consider them as possessing modifying gene(s) or good resistance backgrounds, in a similar response as described by Parlevliet (1975). The absence of transgressive segregants in some populations could be due to classification of the susceptible and/or resistant parents to the most susceptible and/or resistant grouping classes. Application of Tukey's statistics, HSD and making a type II error might have caused grouping of progenies into the parental classes. Additionally, it should be considered as a fact that even if there is no difference between the means of two individuals statistically, it may not necessarily reject the idea that there might be biological difference between them. For example, if the difference between SAUDPC's of 0 and 8 is not statistically significant, a plant with symptoms different from that with a compatible reaction to the pathogen is also not significantly different. Similar conclusion can be drawn when the numbers of segregants are high and no genetic ratio can explain the pattern of segregation considering the fact that only few genes were involved in S-SR. In this study depending on the test location/years/population deviation from the disease reactions of susceptible parent ± (std/HSD) statistically resulted in identifications of variable number of segregants. However, this might not necessarily be in accordance with actual/biological differences among RILs and their respective parents. Hence, over/under estimation of related genetic ratios due to differences among test environments and/or GxE interactions, and also possible statistical errors involved in the data analyses can be addressed as sources of such contradictions.

All ICARDA/CIMMYT generation trials were subjected to diallel analysis and the total variance of each was partitioned into its components. The analyses confirmed the significance of GCA and SCA effects in all cases indicating the presence of additive and non-additive effects of gene(s) governing S-SR. The results further revealed that GCA was the major portion of the total genetic variance for slow-scalding resistance. Therefore, it was concluded that additive gene effects were more important than the non-additive and reciprocals. Similar findings were previously reported for barley leaf rust (Parlevliet, 1978).

Discrepancies among results could be due to application of different methodologies (Baker, 1978). For example, in Griffing's method I and III, diallel crosses are expected to be partitioned into different values of GCA and SCA, where no parents are included in combining ability analysis of method III in order to obtain unbiased estimates of genetic parameters (Das and Griffey, 1994; Navabi *et al.*, 2003). Additionally, when there is no epistasis nor linkage of genes, Hayman's method could not only result in a similar interpretation of additive effects as in Griffing's method I, but could also generate more detailed information on both non-additive and reciprocal/maternal effects (Baker, 1978). In the present study, different methods of diallel analyses were used to verify the results obtained. A good agreement was found between various methods of analyses performed. This agreement was confined to the significance levels since there were differences between the magnitude of components for different methods.

An estimation of genetic components of inheritance from a diallel analysis always requires some assumptions to be considered. They are (1) diploid segregation, (2) homozygous parents, (3) gene frequencies of 0.5 at all segregation loci, (4) the independent distribution of genes in diallel parents, and (5) no non-allelic interaction (no epistasis). Of these assumptions, the last two are difficult to accept in practice (Baker, 1978), where the latter is even not likely to be biologically realistic (Gilbert, 1958). Acceptability of assumptions requires proof when dealing with quantitative traits (Sokal, 1977). Failure to meet these assumptions could cast a serious doubt on the findings from diallel analysis when studying the genetics of self-pollinating crops (Sokal, 1977) and the interpretation of additive and dominance genetic variances (Hayman, 1954a). For example, the estimate of the average degree of dominance could be increased or even decreased when the genes in the parents are not independent (Hayman, 1954a). In the present study, the deviation of the slope of the V_r-W_r line from unity was significant indicating that this diallel set did not satisfy the hypotheses assumed for a diallel analysis, which indicated that epistatic effects should also explain a portion of the total genetic variance. However, the results obtained from different diallel analyses provided us with useful information regarding the relative importance of genetic effects governing S-SR. It has been stated that even if the requirements for an accurate diallel analysis are not met, it could provide a researcher with useful information about combining ability mean squares and effects necessary to assess the potential of a cross in different breeding programs (Baker, 1978).

The slow-scalding parents and the lines prepared in the study could be useful as parents in a crossing program for scald resistance. The ICARDA/CIMMYT slow-scalding parents themselves have not been characterized as barleys with good quality characteristics and might have undesirable quality/agronomic features genetically linked to their genes governing slow-scalding. RILs of such resistant genotypes and Stander as a malting barley could be likely candidates for use in facilitating the improvement of scald resistance in malting barleys. On the other hand, obtaining a genotype with a high level of scald resistance and acceptable agronomic and quality characteristics could be achieved more easily by transferring/pyramiding resistance genes with additive effects into susceptible commercial materials using the backcross method (Ehdaie and Baker, 1999). Therefore, the high levels of heritability coupled with the major role of additive effects in

S-SR could facilitate achieving high-quality barley breeding lines with a high level of field resistance. It could also facilitate selection procedures in early segregating populations and increase the genetic gain. Using GCA and SCA with lowest negative values, selections can be made to choose the best parents for gene pyramiding and durable resistance breeding.

Earlier reports have not indicated the number of genes nor the heritability of slow-scalding. The results of the present study revealed that the S-SR in the three ICARDA/CIMMYT slow-scalding parents was controlled by 1 to 3 genes with additive effects. Since the parental lines had no pedigree in common and transgressive segregation was observed in the studied resistant parent intercrosses, it was suggested that the parents may possess different resistance genes. In the present study, less numbers of effective genes were found for the ICARDA/CIMMYT populations studied in Lacombe. It was rationalized that while a number of resistance genes could be effective due to absence of their matching virulence genes in the population of a pathogen in a given location, the presence of some virulent races of the pathogen may decrease the effectiveness of resistance in another location. Consequently, changes in the estimates of genetic parameter of a given cross across different generations/locations trial could be due to changes in the number of effective factors governing the resistance in respective environments. One to four minor genes were also estimated to be involved in the S-SR of CDC Dolly where the bimodal distribution of DH and DT populations in Canada could suggest that in addition to the minor genes at least one major gene may also contribute towards resistance in that genotype. The heritability of S-SR was found to be high in all instances. However, slight differences were observed among the estimates of different traits in the test generations/locations. Estimates of heritability for the same trait in the same crop may differ widely; however, they can provide breeders with useful information about the selection potential of breeding materials.(Fehr, 1987).

It was concluded that heritability of slow-scalding resistance in the studied population was operating on a quantitative basis. Also, it was mainly under the control of several genes with additive actions, where dominance and additive by additive epistatic interaction were also contributing to S-SR. Since the ICARDA/CIMMYT parental used in this study has consistently performed with a stable level of field resistances at Toluca (according to the field data since 1986, Dr. F. Capettini, ICARDA/CIMMYT Barley Breeding Program, personal communication), S-SR in their progenies could be considered a long-lasting type of resistance. More studies using barley crosses with more and different slow-scalding parents are needed in order to achieve a better understanding of the inheritance of slow-scalding. Mean generation analyses using a larger number of slow-scalding lines and their F1s, F2s, F3s, and backcrosses are suggested to investigate epistasis in a more precise way.

6.5. Tables and Figures

Table 6-1. Mean and standard deviation (std) of standardized area under the disease progress curve (SAUDPC, 0-100), and terminal severity (TS, 0-9) of parental lines, and the mean of SAUDPC for their crosses including reciprocals tested at different locations using Tukey's HSD values.

		Generation_Location‡							
Parents†		**F1_Edm**	**F2_Edm**	**F3_Edm**	**F5_Edm**	**F2_Lac**	**F3_Lac**	**F2_Tol**	**F3_Tol**
TS	Stander	7.8±0.5	7.6±0.8	6.7±0.8	7.2±0.9	8.7±0.6	6.5±0.5	6.8±0.3	6.2±0.3
	UNA 80	4.1±0.9	4.0±1.4	1.7±1.2	4.8±1.5	4.7±0.8	4.0±1.3	2.3±1.2	4.0±0.5
	Zavila	0.7±0.6	0.5±0.2	0.3±0.6	2.4±1.1	0.6±0.7	0.2±0.4	4.0±0.8	4.3±0.9
	CI1240/F.	3.3±1.0	2.2±1.7	0.7±0.6	3.0±1.1	4.7±0.6	3.8±0.3	3.5±1.0	3.7±0.8
SAUDPC	Stander (S)	89.3±5.3	85.8±9.1	89.4±10.7	85.7±5.0	93.1±11.5	86.4±6.6	80.7±25.9	84.3±13.2
	UNA 80 (U)	45.7±7.1	42.6±17.3	23.5±10.4	45.5±1.4	49.9±12.5	53.2±17.6	28.3±10.6	42.7±8.9
	Zavila (Z)	1.3±1.2	0.6±0.5	4.0±7.0	29.5±2.6	6.2±6.8	2.2±5.4	42.5±12.1	42.9±6.8
	CI1240/F.(C)	20.9±10.0	15.4±16.6	2.9±2.5	36.3±2.0	51.9±13.0	51.0±3.8	35.3±4.4	36.1±3.0

		Generation_Location							
Crosses (SAUDPC)		**F1_Edm**	**F2_Edm**	**F3_Edm**	**F5_Edm**	**F2_Lac**	**F3_Lac**	**F2_Tol**	**F3_Tol**
Susceptible x resistant									
	SxU	50.47	63.83	46.34	63.78*	52.52	58.50	57.13	43.33
	UxS	50.53	68.15	48.51	52.21*	56.85	57.24	61.89	52.51
	SXZ	23.13	45.51*	28.28	47.03	26.94	29.47	44.32	63.45
	ZxS	19.70	36.90*	30.65	41.53	22.42	23.02	56.97	62.79
	SxC	35.83*	53.20*	19.94	40.07*	73.09	74.65*	41.48	39.52
	CxS	41.77*	57.62*	30.17	48.10*	70.09	56.72*	41.43	48.00
Resistant x resistant									
	UxZ	9.73	15.68*	6.14	36.44	10.24	11.30	30.55	29.59
	ZxU	9.13	21.56*	5.60	41.22	6.46	19.01	41.62	34.60
	UxC	9.90*	19.00	6.62	39.72	34.69	31.46	21.64	18.71
	CxU	16.70*	19.75	5.41	40.76	34.47	31.13	21.53	18.67
	ZxC	0.50	11.78	4.11	34.46	15.09	14.73	33.46	28.79
	CxZ	5.90	11.50	4.00	30.54	13.23	15.78	31.67	27.98
HSD (P=0.05)		5.56	4.34	15.35	5.98	16.49	17.10	20.43	15.05

† CI1240/F. represents CI1240/FOMA//CI6239.15D. HSD values and indicates minimum significant difference between means within a column for SAUDPC.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively.

* Reciprocal crosses with significant differences at P<0.05.

Table 6-2. Correlation analyses (n = 16) for standardized area under the disease progress curve (SAUDPC) and terminal severity (TS) of all generation trials studied.

	Generation/Location†			
	F1_Edm	**F2_Edm**	**F3_Edm**	**F5_Edm**
F1_Edm	**0.87****	0.94**	0.84**	0.90**
F2_Edm	0.93**	**0.99****	0.92**	0.92**
F3_Edm	0.92**	0.92**	**0.99****	0.92**
F5_Edm	0.88**	0.90**	0.89**	**0.98****

	Generation/Location‡			
	F2_Lac	**F3_Lac**	**F2_Tol**	**F3_Tol**
F2_Lac	**0.99****	0.97**	-	-
F3_Lac	0.97**	**0.99****	-	-
F2_Tol	-	-	**0.98****	0.85**
F3_Tol	-	-	0.87**	**0.96****

† Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively. Diagonal bold coefficients show the correlation between SAUDPC and TS for each generation trial whereas the off-diagonal coefficients represent the relationships among generations within each location for SAUDPC (above the diagonal) and TS (below the diagonal).

** Significant at P<0.01.

Table 6-3. Mid-parent values (MP), deviation from mid-parents (DMP), skewness (SKW), and standard error (SE) of standardized area under the disease progress curve (SAUDPC) for all generation trials.

Crosses†		Generation_Location‡							
		F1_Edm	F2_Edm	F3_Edm	F5_Edm	F2_Lac	F3_Lac	F2_Tol	F3_Tol
SxR									
SxU	MP	67.50	64.20	56.45	65.60	71.50	69.80	54.50	63.50
	DMP	-17.02	1.75	-9.03	-7.62	-16.79	-11.93	5.01	-15.59
	SKW	-0.63	-1.00	0.34	0.26	-0.57	-1.30	-0.23	0.26
	SE	0.60	1.47	2.51	1.01	2.30	1.94	1.73	2.02
SxZ	MP	45.30	43.20	46.70	57.60	49.65	44.30	61.60	63.60
	DMP	-23.88	-2.06	-17.46	-13.32	-24.98	-18.26	-11.10	-0.46
	SKW	0.02	0.13	0.93	0.07	0.80	0.49	0.17	-0.36
	SE	0.92	1.38	2.26	1.34	2.28	2.56	1.55	1.53
SxC	MP	55.10	50.60	46.15	61.00	72.50	68.70	58.00	60.20
	DMP	-16.28	4.80	-21.22	-16.90	-0.92	-3.00	-16.58	-16.44
	SKW	-0.99	-0.65	1.06	-1.21	-0.07	-1.13	0.12	0.22
	SE	1.10	1.60	1.56	0.78	0.97	1.71	1.75	2.20
RxR									
UxZ	MP	23.50	21.60	13.75	37.50	28.05	27.70	35.40	42.80
	DMP	-14.08	-3.00	-8.08	1.34	-19.70	-12.74	0.56	-10.68
	SKW	0.42	1.24	1.84	0.18	2.44	1.37	0.41	-0.16
	SE	0.67	1.20	0.87	1.19	1.43	1.81	1.49	1.35
UxC	MP	33.30	29.00	13.20	40.90	50.90	52.10	31.80	39.40
	DMP	-20.00	-9.59	-7.29	-0.64	-16.32	-20.77	-10.22	-20.71
	SKW	0.40	1.33	0.94	0.15	0.28	0.20	0.40	0.53
	SE	0.50	1.50	0.67	0.95	1.86	2.15	0.95	1.11
ZxC	MP	11.10	8.00	3.45	32.90	29.05	26.60	38.90	39.50
	DMP	-7.88	3.65	0.28	-0.37	-14.93	-11.52	-6.49	-11.09
	SKW	1.56	1.50	1.64	0.45	1.51	0.90	0.29	0.16
	SE	0.46	1.06	0.60	0.88	1.53	1.45	1.39	1.27

† SxR and RxR represent resistant x susceptible crosses and resistant parent intercrosses, respectively. S,U,Z and C are parental lines as explained in Table 6-1.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively.

Table 6-4. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for susceptible x resistant crosses (SxR).

		No. of lines with disease severity												Transg[§]		%Transg	
Cross[†]	Gen/Loc[‡]	10	20	30	40	50	60	70	80	90	More	Total	Range	R	S	R	S
SxU	F2Edm	2	3	4	8	9	23	33	47	25	8	162	95.9	16	8	0.10	0.05
	F3Edm	4	10	12	18	10	12	10	6	8	6	96	95.5	2	0	0.02	0.00
	F5Edm	0	0	0	4	17	51	22	13	4	0	111	53.9	4	0	0.04	0.00
	F2Lac	4	6	10	6	10	16	28	10	10	4	104	99.7	20	0	0.19	0.00
	F3lac	8	0	2	10	6	34	32	16	4	2	114	93.1	10	0	0.09	0.00
	F2Tol	0	0	1	7	11	17	18	15	3	0	72	68.9	0	0	0.00	0.00
	F3Tol	0	3	6	14	18	15	7	5	4	0	72	69.1	8	0	0.11	0.00
SxZ	F2Edm	18	35	41	37	37	38	28	19	11	2	266	94.0	0	2	0.00	0.01
	F3Edm	21	19	19	11	15	2	5	4	2	1	99	94.8	0	0	0.00	0.00
	F5Edm	1	1	20	24	25	28	15	1	1	0	116	82.7	3	0	0.03	0.00
	F2Lac	32	28	18	5	9	4	14	2	1	0	113	99.7	0	0	0.00	0.00
	F3lac	48	9	10	13	1	15	13	4	0	1	114	93.1	0	0	0.00	0.00
	F2Tol	1	9	13	24	33	26	27	11	7	5	156	88.1	21	0	0.13	0.00
	F3Tol	0	1	6	14	13	20	34	29	20	7	144	87.2	7	1	0.05	0.01
SxC	F2Edm	4	10	14	12	19	29	36	35	13	1	173	90.2	6	1	0.03	0.01
	F3Edm	12	22	30	18	8	2	1	1	0	0	94	73.1	0	0	0.00	0.00
	F5Edm	1	0	5	25	60	26	1	0	0	0	118	61.6	6	0	0.05	0.00
	F2Lac	0	0	0	0	2	16	32	32	30	4	116	43.6	0	0	0.00	0.00
	F3lac	2	4	0	4	4	32	34	22	10	10	122	99.7	6	0	0.05	0.00
	F2Tol	0	5	15	14	15	14	7	2	0	0	72	63.6	2	0	0.03	0.00
	F3Tol	2	5	14	14	8	14	10	3	2	0	72	82.0	9	0	0.13	0.00

† S,U,Z and C are parental lines as explained in Table 6-1.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively. § Number of resistant (R), and susceptible (S) transgressive segregants exhibiting lower (mean-HSD) and higher (mean+HSD) SAUDPC values than those of the respective parents, respectively.

Table 6-5. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for resistant parent intercrosses.

		No. of lines with disease severity												Transg§		%Transg	
Cross†	Gen/Loc‡	10	20	30	40	50	60	70	80	90	More	Total	Range	R	S	R	S
UxZ	F2Edm	101	37	20	22	11	8	1	1	0	0	201	80.1	0	11	0.00	0.05
	F3Edm	73	14	5	1	1	0	0	0	0	0	94	40.4	50	1	0.53	0.01
	F5Edm	1	3	15	38	23	13	1	2	0	0	96	76.2	7	14	0.07	0.15
	F2Lac	63	24	15	2	1	1	1	1	0	0	108	81.0	0	2	0.00	0.02
	F3lac	60	20	12	5	3	6	3	0	0	0	109	66.5	0	0	0.00	0.00
	F2Tol	12	29	22	31	22	17	6	4	1	0	144	81.7	5	8	0.03	0.06
	F3Tol	18	22	19	26	35	21	3	0	0	0	144	65.5	54	7	0.38	0.05
UxC	F2Edm	61	30	26	12	8	6	2	5	0	0	150	79.1	65	13	0.43	0.09
	F3Edm	70	26	4	0	0	0	0	0	0	0	100	24.3	42	0	0.42	0.00
	F5Edm	0	1	8	39	26	12	2	0	0	0	88	55.5	9	9	0.10	0.10
	F2Lac	6	24	24	16	26	5	11	2	2	0	116	87.3	59	4	0.51	0.03
	F3lac	28	8	32	20	0	16	10	6	0	0	120	79.8	69	6	0.58	0.05
	F2Tol	24	46	40	24	10	0	0	0	0	0	144	49.9	18	0	0.13	0.00
	F3Tol	54	28	34	24	2	4	0	0	0	0	146	54.8	84	0	0.58	0.00
ZxC	F2Edm	108	31	15	13	6	3	1	0	0	0	177	62.0	0	39	0.00	0.22
	F3Edm	81	16	2	0	0	0	0	0	0	0	99	27.2	57	0	0.58	0.00
	F5Edm	0	5	44	30	19	4	0	0	0	0	102	38.6	18	19	0.18	0.19
	F2Lac	43	40	16	7	3	1	4	0	0	0	114	68.6	0	2	0.00	0.02
	F3lac	48	29	29	5	6	3	1	0	0	0	121	66.5	48	0	0.40	0.00
	F2Tol	16	20	30	34	21	14	6	3	0	0	144	77.5	23	7	0.16	0.05
	F3Tol	20	26	33	30	21	11	3	0	0	0	144	63.4	52	5	0.36	0.03

† S,U,Z and C are parental lines as explained in Table 6-1.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively. § Number of resistant (R), and susceptible (S) transgressive segregants exhibiting lower (mean-HSD) and higher (mean+HSD) SAUDPC values than those of the respective parents, respectively.

Table 6-6. Mean squares for standardized area under the disease progress curve (SAUDPC) obtained from Hayman's and Griffing's diallel analyses.

		Generation/Location‡							
S.o.V†	df	F1_Edm	F2_Edm	F3_Edm	F5_Edm	F2_Lac	F3_Lac	F2_Tol	F3_Tol
Replications	2	0.2	2.5	0.8	3.3	43.2	15.7	164.8 *	66.7
genotypes	15	1712.5 **	1845.7 **	1673.9 **	576.2 **	2098.3 **	1794.4 **	746.3 **	907.8 **
Hayman (1954)									
a	3	7439.1 **	8930.9 **	7568.2 **	2324.7 **	9297.8 **	7916.9 **	3027.4 **	3725.9 **
b	6	530.9 **	111.9 **	371.6 **	211.1 **	580.2 **	421.1 **	274.9 **	361.0 **
b1	1	2457.7 **	5.0	986.0 **	351.4 **	2192.4 **	1529.8 **	376.6 **	1404.9 **
b2	3	51.7 **	60.6 **	181.4 **	273.1 **	215.5 **	50.6	213.4 **	231.6 **
b3	2	286.5 **	242.3 **	349.8 **	47.8 **	321.1 **	422.6 **	316.4 **	33.1
c	3	57.8 **	4.4	39.6	52.6 **	18.3	128.5 *	124.4	49.0
d	3	3.6	69.3 **	18.8	81.4 **	14.7	84.2	29.9	42.2
Griffing (1956)									
GCA (MI)	3	7439.1 **	8930.9 **	7568.2 **	2324.7 **	9297.8 **	7916.9 **	3027.4 **	3725.9 **
SCA (MI)	6	530.9 **	111.9 **	371.6 **	211.1 **	580.2 **	421.1 **	274.9 **	361.0 **
Crosses (C)	11	2013.9 **	2967.1 **	1819.2 **	234.5 **	3571.8 **	2806.0 **	1086.1 **	1508.1 **
GCA (MIII)	3	3165.6 **	4783.7 **	2798.8 *	693.9 **	5739.0 **	4395.0 **	1599.1 *	2491.4 **
SCA (MIII)	2	286.5 **	242.3 **	349.8 **	47.8 **	321.1 **	422.6 **	316.4 **	33.0
RE	6	30.7 **	36.9 **	29.2	67.0 **	16.5	106.4 **	77.2	45.6
Parent (P)	3	4325.2 **	4207.8 **	4950.6 **	1904.0 **	3775.0 **	3572.8 **	1641.7 **	1466.0 **
P vs. C	1	2457.7 **	5.0	986.1 **	351.5 **	2191.9 **	1529.8 **	376.6 **	1405.1 **
Error	30	3.4	2.0	25.4	3.9	29.4	31.6	45.1	24.5

† According to Hayman (1954), a and b represent additive and nonadditive effects, where b1 represents directional dominance effect; a significant b2 implies asymmetry of gene distribution, and b3 is part of the dominance deviation that is unique to corresponding F population. Also, c and d represent reciprocal effects where c detects the average maternal effects of each parental line and d describes the reciprocal differences not ascribed to c. According to Griffing (1956), GCA and SCA represent general and specific combining abilities, respectively. In both methods I and III, reciprocal crosses are included in the diallel analyses. In contrast, these methods are different in terms of including parents into the analysis, where parents are eliminated in the later method.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively.

*,** Significant at $P<0.05$ and $P<0.01$, respectively.

Table 6-7. Combining ability values of standardized area under the disease progress curve (SAUDPC) for all generation trials.

Effect[†]	Line/Cross[‡]	Generation_Location[§]							
		F1_Edm	F2_Edm	F3_Edm	F5_Edm	F2_Lac	F3_Lac	F2_Tol	F3_Tol
GCA	Stander (S)	23.09	26.54	25.61	13.47	22.43	20.54	16.17	17.65
	UNA 80 (U)	2.82	1.09	-1.52	1.09	-1.71	0.85	-5.55	-6.76
	Zavila (Z)	-18.07	-17.53	-11.37	-8.28	-25.22	-23.74	-1.45	-0.51
	CI1240/F.(C)	-7.84	-10.10	-12.72	-6.28	4.50	2.35	-9.17	-10.38
SE_{GCA}		0.53	0.64	1.46	0.57	1.56	0.94	1.94	1.43
SCA	UxS	-2.36	2.78	1.09	-1.12	-4.63	-2.24	6.98	-5.09
	ZxS	-10.52	-3.37	-7.02	-5.46	-11.10	-9.02	-5.98	3.85
	CxS	-3.34	3.41	-10.05	-7.65	6.08	4.37	-7.46	-5.64
	ZxU	-2.24	-0.50	-3.42	1.47	-3.30	-0.41	1.17	-2.76
	CxU	-8.57	-7.14	-1.97	0.88	-6.76	-10.37	-5.60	-6.25
	CxZ	2.22	3.72	5.90	2.51	-3.69	-1.81	1.28	-2.84
$SE1_{SCA}$ *		0.92	1.11	2.52	0.98	2.71	1.62	3.36	2.47
$SE2_{SCA}$		0.75	0.91	2.06	0.80	2.21	1.32	2.74	2.02

† GCA and SCA represent general and specific combining abilities, respectively. SE represents standard error where $SE1_{SCA}$ and $SE2_{SCA}$ are given to compare the differences between effects of two crosses having one and no parent line in common, respectively.

‡ CI1240/F. represents CI1240/Foma// CI16239.15D.

§ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively.

Table 6-8. Genetical components of variance in different generation trials studied using different diallel analyses methods.

	Generation_ Location‡							
Component†	**F1_Edm**	**F2_Edm**	**F3_Edm**	**F5_Edm**	**F2_Lac**	**F3_Lac**	**F2_Tol**	**F3_Tol**
With parents								
Crosses	569.66	614.55	549.51	190.76	689.62	587.61	233.75	294.44
σ^2_{GCA}	289.52	367.81	300.97	88.73	365.00	313.57	115.42	141.28
σ^2_{SCA}	108.23	22.54	71.02	42.50	112.98	79.91	47.15	69.02
σ^2_r	4.52	5.81	0.63	10.52	2.15	12.46	5.35	3.52
D	1440.61	1401.94	-	-	1248.31	-	532.18	-
H1	359.77	83.01	-	-	398.22	-	181.29	-
H2	351.86	73.33	-	-	368.42	-	155.12	-
Contribution								
GCA	0.837	0.963	0.894	0.770	0.864	0.872	0.815	0.796
SCA	0.156	0.029	0.105	0.184	0.134	0.111	0.166	0.194
RE	0.007	0.008	0.001	0.046	0.003	0.017	0.019	0.010
D/H1	4.00	16.89	-	-	3.13	-	2.94	-
Baker ratio	0.84	0.97	0.89	0.81	0.87	0.89	0.83	0.80
Heritability	0.87	0.98	-	0.84	0.88	-	0.82	-
Without parents								
Crosses	309.25	455.96	277.75	77.53	543.46	441.26	178.28	232.99
σ^2_{GCA}	239.84	378.45	204.09	53.84	451.49	331.03	106.89	204.86
σ^2_{SCA}	47.04	40.23	56.70	7.65	53.15	68.82	52.58	3.61
σ^2_r	4.44	5.99	3.27	10.85	2.39	16.11	12.71	5.70
Additive (Add)	479.67	756.90	-	-	902.99	-	213.79	-
Dominance(Dom)	47.04	40.23	-	-	53.15	-	52.58	-
Contribution								
GCA	0.903	0.942	0.872	0.853	0.942	0.886	0.766	0.978
SCA	0.089	0.050	0.121	0.061	0.055	0.092	0.188	0.009
RE	0.008	0.007	0.007	0.086	0.002	0.022	0.046	0.014
Add/Dom	10.20	18.82	-	-	16.99	-	4.07	-
Baker ratio	0.91	0.95	0.88	0.93	0.94	0.91	0.80	0.99
Heritability	0.91	0.95	-	0.93	0.94	-	0.80	-

† GCA, SCA, and RE represent general (σ^2_{gca}) and specific (σ^2_{gca}) combining abilities, and reciprocal effects (σ^2_r), respectively. D, and H1 and H2 are additive, and dominance components of variance (Mather and Jinks, 1974). "Contribution" shows proportion of each effect relative to the total variance. A/D represent the ratio of Additive/Dominance whereas Baker ratio show $2\sigma^2_{gca}/(2\sigma^2_{gca} + \sigma^2_{sca})$.

‡ Edm, Lac, and Tol represent Edmonton, Lacombe, and Toluca, respectively.

Table 6-9. Number of genes estimated for slow-scalding resistance in the populations of crosses between the susceptible cultivar Stander and three CIMMYT/ICARDA parental lines.

		Gene No.[‡]		
Cross[†]	**Location**	**GNF1 (F1 + F2 data)**	**GNF 2 (F3 data)**	**GNF 3 (F5 data)**
UNA 80 x Stander	Edmonton	3.3	2.7	3.1
	Lacombe	0.9	0.8	-
	Toluca	2.7	2.5	-
Zavila x Stander	Edmonton	2.7	3.2	2.8
	Lacombe	2.2	2.4	-
	Toluca	3.0	2.6	-
CI1240/F. X Stander	Edmonton	2.4	2.2	2.0
	Lacombe	2.1	1.5	-
	Toluca	2.9	3.3	-

† CI1240/F. represents CI1240/Foma// CI16239.15D.

‡ GNF: Gene number formula according to Bjarko and Line (1988).

Table 6-10. Mean squares for the traits studied in (A) population CDC Dolly 6 x RFLP Harrington and (B) population CDC Dolly x TR 251.

			Mean square‡					
Location/ Year	**S.o.V†**	**df**	**FS**		**AS**		**TS**	
Edmonton 2000	**Rep**	3	9.87		14.82		0.11	
	Block (Rep)	40	2.60		2.93		0.04	
	Gen	201	434.28	**	683.36	**	17.65	**
	Error§	563	94.61	a	92.22	a	1.88	a
Edmonton 2001	**Rep**	3	4.78		7.38		0.16	
	Block (Rep)	39	2.84		3.52		0.07	
	Gen	201	717.22	**	947.09	**	20.94	**
	Error	636	91.60	a	99.69	a	2.07	a
Lacombe 2000	**Rep**	2	0.90		0.89		<.01	
	Block (Rep)	30	2.57		<.01		0.07	
	Gen	201	625.46	**	321.64	**	9.58	**
	Error	372	61.99	b	29.98	c	1.08	b
Lacombe 2001	**Rep**	2	1.34		11.04		0.06	
	Block (Rep)	427	9.24	c	74.29	b	0.63	c
	Gen	29	0.50		2.46		<.01	
	Error	201	60.43	**	473.23	**	3.51	**

			Mean square					
Location/ Year	**S.o.V**	**df**	**FS**		**AS**		**TS**	
Edmonton 2000	**Rep**	2	0.79		1.42		0.01	
	Block (Rep)	32	0.02		<.01		<.01	
	Gen	221	205.30	**	371.02	**	14.68	**
	Error	464	25.31	b	39.01	b	1.14	b
Edmonton 2001	**Rep**	2	0.76		1.76		0.03	
	Block (Rep)	32	6.78		5.93		0.04	
	Gen	221	689.45	**	833.26	**	16.08	**
	Error	464	104.62	a	105.61	a	1.76	a

† S.o.V: Source of variation, Rep: Replicate, Gen: Genotype.

‡ FS: Final disease severity (1-100). AS: Standardized area under the disease progress curve (1-100). TS: Terminal severity (1-9).

§ Error mean squares with the same letter are not significantly different according to Bartlett's test (Steel *et al.*, 1997).

* and ** Significant at P< 0.05 and 0.01, respectively.

Table 6-11. Combined analysis of final disease severity (FS, 1-100), standardized area under the disease progress curve (AS, 1-100), and terminal severity (TS, 1-9) for the population CDC Dolly 6 x RFLP Harrington studied in Edmonton during 2001-2002.

		Mean square		
S.o.V†	df	FS	AS	TES
Env	1	13.0 **	8.8 **	0.46 **
Rep (Env)	6	7.2	11.0	0.13
Block (Env*Rep)	79	2.6	3.1	0.04
Gen	201	652.5 **	989.3 **	21.96 **
Gen x Env	200	11.9 **	11.5 **	0.29 **
Error	1200	93.9	96.7	2.00

† S.o.V: Source of variation, Env: Environment, Rep: Replicate, Gen: Genotype.
** Significant at P< 0.01.

Table 6-12. Parental mean ± standard deviation (std), and the number of transgressive segregants observed for the populations of (A) CDC Dolly 6 x RFLP Harrington and (B) CDC Dolly x TR 251.

		Parental mean ± std		Trans‡		%Trans	
Trait†	Location/ Year	CDC Dolly	Harrington	R	S	R	S
FS	Edmonton 2000	3.9±0.4	26.4±7.5	69	6	0.35	0.03
	Edmonton 2001	5.3±1.8	31.7±3.5	47	22	0.24	0.11
	Edmonton (Combined)	4.4±1.3	28.3±5.5	55	11	0.28	0.06
	Lacombe 2000	20.1±1.8	36.3±3.6	68	50	0.34	0.25
	Lacombe 2001	15.6±2.7	32.7±2.5	73	0	0.37	0.00
	Toluca 2001	45.8±4.1	38.4±5.5	108	19	0.54	0.10
AS	Edmonton 2000	4.4±1.4	31.0±7.8	11	18	0.06	0.09
	Edmonton 2001	5.2±1.0	38.8±9.7	5	14	0.03	0.07
	Edmonton (Combined)	4.7±1.3	35.4±8.7	3	13	0.02	0.07
	Lacombe 2000	25.7±2.0	47.1±2.2	66	4	0.33	0.02
	Lacombe 2001	21.1±8.7	41.8±5.9	4	21	0.02	0.11
	Toluca 2001	40.2±14.1	41.3±7.5	34	52	0.17	0.26
TS	Edmonton 2000	1.5±0.4	5.1±0.6	42	40	0.21	0.20
	Edmonton 2001	2.1±0.8	5.9±0.7	8	56	0.04	0.28
	Edmonton (Combined)	1.8±0.3	5.5±0.6	22	45	0.11	0.23
	Lacombe 2000	4.3±0.6	7.6±0.3	28	37	0.14	0.19
	Lacombe 2001	3.2±0.9	6.2±0.8	9	0	0.05	0.00
	Toluca 2001	7.1±0.7	6.6±0.8	66	42	0.33	0.21

		Parental mean ± std		Trans		Trans	
Trait	Location/ Year	CDC Dolly	TR 251	R	S	R	S
FS	Edmonton 2000	2.7±0.6	23.9±5.7	70	1	0.32	0.00
	Edmonton 2001	3.3±0.8	27.7±3.5	30	51	0.14	0.23
	Lacombe 2000	18.1±0.8	39.2±1.5	19	85	0.09	0.39
	Lacombe 2001	15.7±2.3	27.7±2.7	88	0	0.40	0.00
	Toluca 2001	40.8±4.1	39.2±1.5	154	26	0.70	0.12
AS	Edmonton 2000	4.2±1.6	36.5±5.1	44	34	0.20	0.16
	Edmonton 2001	4.2±1.4	38.8±5.7	2	48	0.01	0.22
	Lacombe 2000	23.7±2.1	41.1±2.2	13	86	0.06	0.39
	Lacombe 2001	21.1±8.7	45.8±5.9	21	31	0.10	0.14
	Toluca 2001	60.2±7.1	51.3±10.0	115	6	0.53	0.03
TS	Edmonton 2000	1.5±0.7	5.2±0.8	1	4	0.00	0.02
	Edmonton 2001	1.9±0.8	5.3±1.5	78	2	0.36	0.01
	Lacombe 2000	3.9±0.8	7.0±0.5	10	51	0.05	0.23
	Lacombe 2001	3.6±0.3	6.5±0.4	21	3	0.10	0.01
	Toluca 2001	8.1±0.3	6.8±0.5	122	15	0.56	0.07

† FS: Final disease severity (1-100). AS: Standardized area under the disease progress curve (1-100). TS: Terminal severity (1-9).

‡ Number of resistant (R), and susceptible (S) transgressive segregants exhibiting lower (mean-std) and higher (mean+std) disease severity than those of the respective parents, respectively.

Table 6-13. Mean, standard deviation, heritability, and the number of genes for the populations of (A) CDC Dolly 6 x RFLP Harrington and (B) CDC Dolly x TR 251.

Characteristic[†]	Location/ Year	Trait[‡] FS	AS	TS
Mean±Std	Edmonton 2000	11.4±10.5	20.5±13.2	4.7±2.1
	Edmonton 2001	16.9±13.1	25.4±15.2	5.7±2.2
	Edmonton (Combined)	14.2±13.1	22.3±13.5	5.2±2.1
	Lacombe 2000	29.0±11.1	30.9±10.4	6.0±1.8
	Lacombe 2001	17.8±4.3	32.8±12.1	4.9±1.0
	Toluca 2001	30.8±13.4	39.3±13.4	6.4±1.5
Heritability	Edmonton 2000	0.78	0.87	0.89
	Edmonton 2001	0.87	0.89	0.90
	Edmonton (Combined)	0.87	0.91	0.92
	Lacombe 2000	0.90	0.91	0.89
	Lacombe 2001	0.85	0.84	0.82
Gene No.	Edmonton 2000	2.9	2.7	2.7
	Edmonton 2001	2.5	3.9	3.2
	Edmonton (Combined)	3.4	3.7	3.6
	Lacombe 2000	4.2	4.2	3.0
	Lacombe 2001	3.5	4.4	4.4

Characteristic	Location/ Year	Trait FS	AS	TS
Mean±Std	Edmonton 2000	12.1±8.0	22.7±17.8	4.8±1.3
	Edmonton 2001	17.5±15.1	27.3±16.6	3.9±2.3
	Lacombe 2000	29.4±7.3	38.4±12.4	6.2±1.7
	Lacombe 2001	18.1±4.9	30.2±18.0	5.4±1.2
	Toluca 2001	29.6±12.0	41.4±12.5	6.0±1.4
Heritability	Edmonton 2000	0.88	0.89	0.92
	Edmonton 2001	0.85	0.87	0.89
Gene No.	Edmonton 2000	4.4	4.5	2.9
	Edmonton 2001	3.5	4.5	2.8

† Heritability and gene numbers are only given for those trials with replications. Gene numbers were estimated according to Bjarko and Line (1988).

‡ FS: Final disease severity (1-100). AS: Standardized area under the disease progress curve (1-100). TS: Terminal severity (1-9).

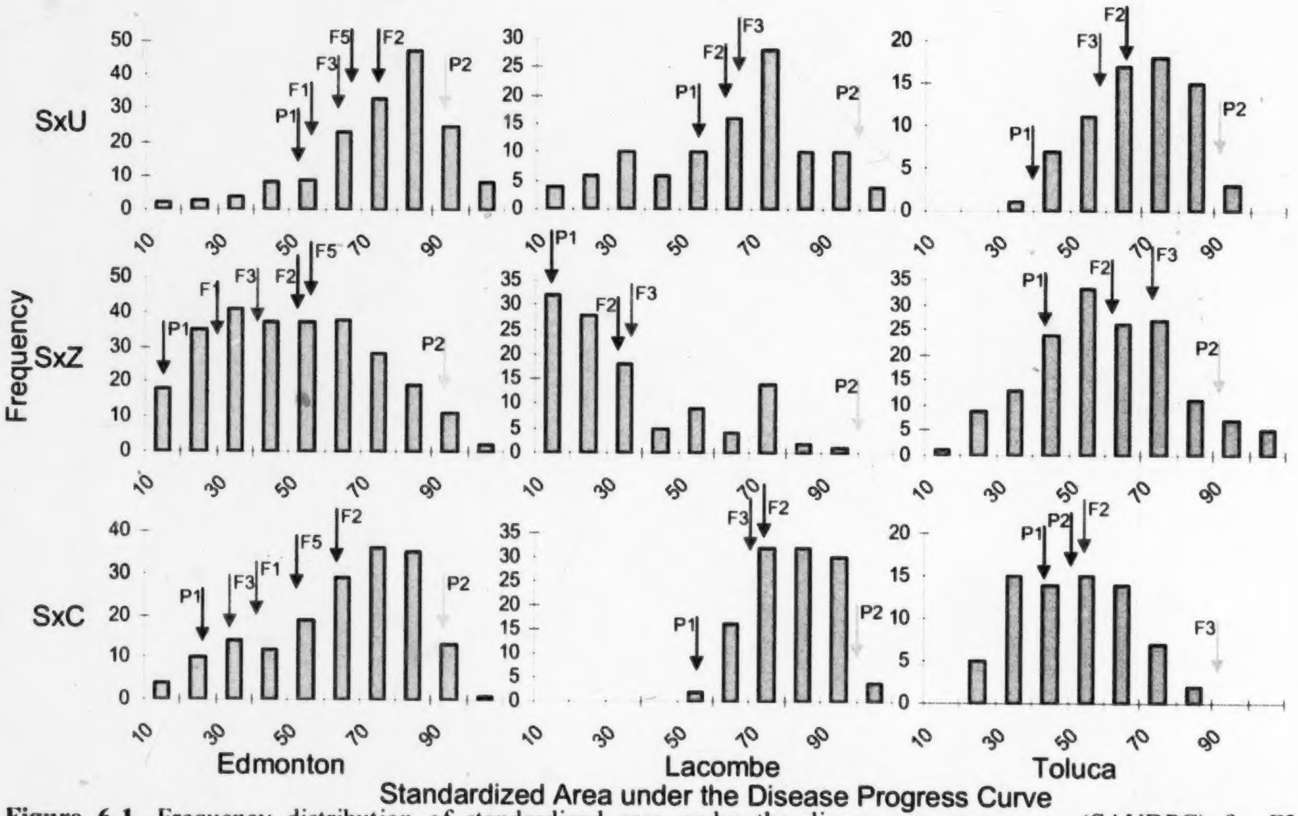

Figure 6-1. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for F2 generations of all SxR crosses/locations, where dark and light green, red, blue, purple and yellow arrows represent the SAUDPC mean values for the most resistant and susceptible parents in F1, F2, F3, and F5 generations, respectively.

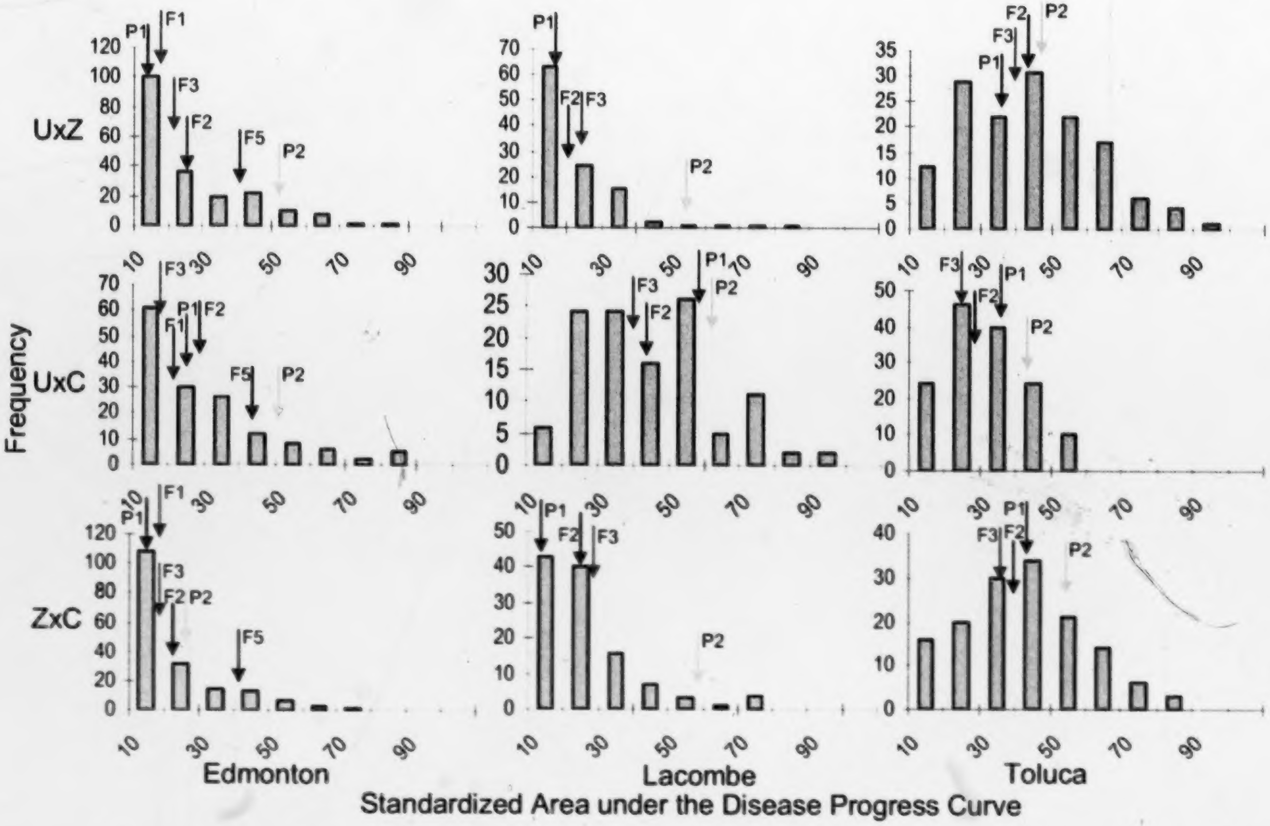

Figure 6-2. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for F2 generations of all resistant parent intercrosses/locations, where dark and light green, red, blue, purple and yellow arrows represent the SAUDPC mean values for the most resistant and susceptible parents in F1, F2, F3, and F5 generations, respectively.

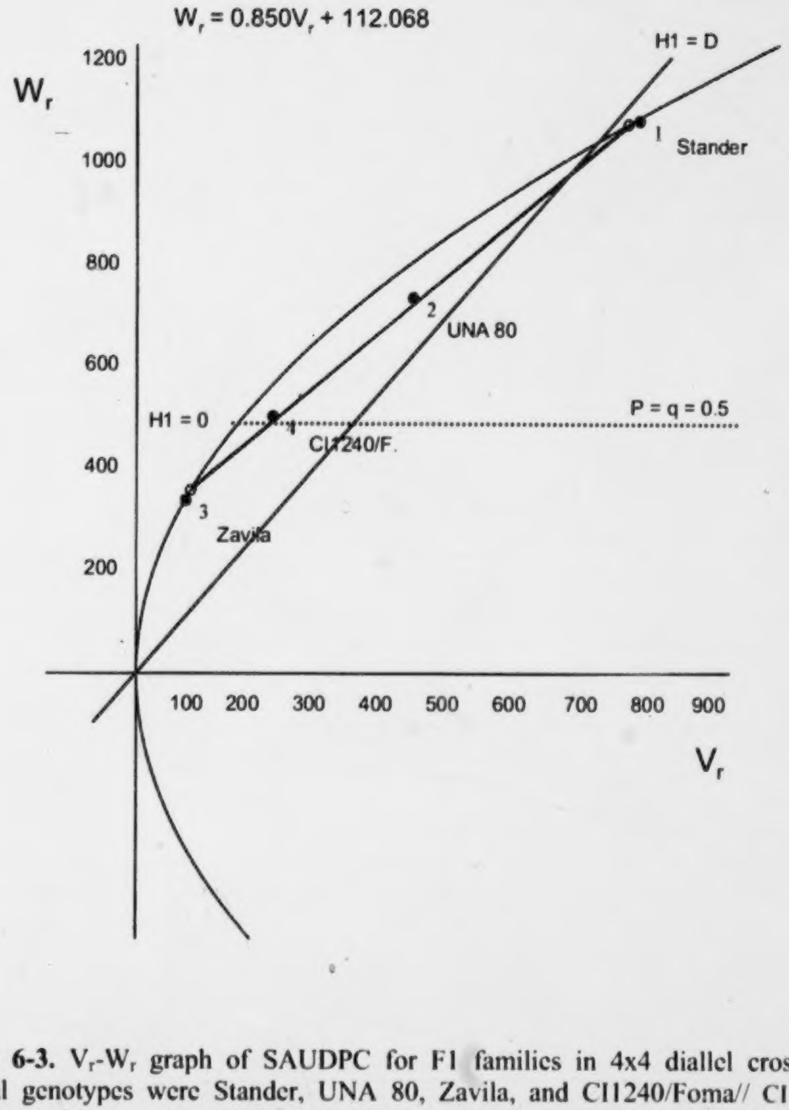

Figure 6-3. V_r-W_r graph of SAUDPC for F1 families in 4x4 diallel crosses where parental genotypes were Stander, UNA 80, Zavila, and CI1240/Foma// CI16239.15D. Note that red line represents the slope of unity where dominance (H1) and additive (D) gene effects are theoretically considered to be equal. Dotted blue line shows the area around which the frequency of dominant (p) and recessive (q) alleles/genes are equal whereas the frequency of p is increased for parents with lower W_r values.

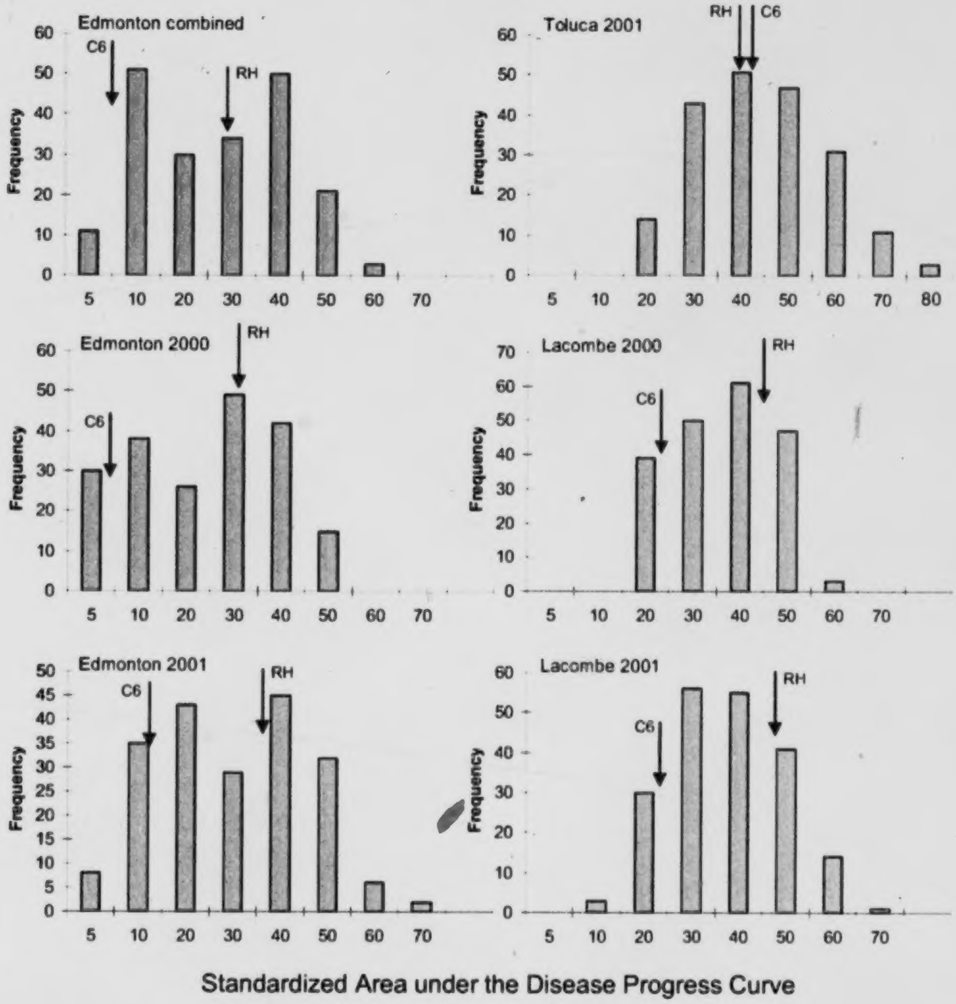
Edmonton combined
Toluca 2001
Edmonton 2000
Lacombe 2000
Edmonton 2001
Lacombe 2001

Figure 6-4. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for scald severity of 200 recombinant inbred lines of the population CDC Dolly 6 and RFLP Harrington studied in Edmonton, Lacombe, Canada, and Toluca, Mexico. Arrows show the SAUDPC means for the parental lines (C6: CDC Dolly 6; RH: RFLP Harrington).

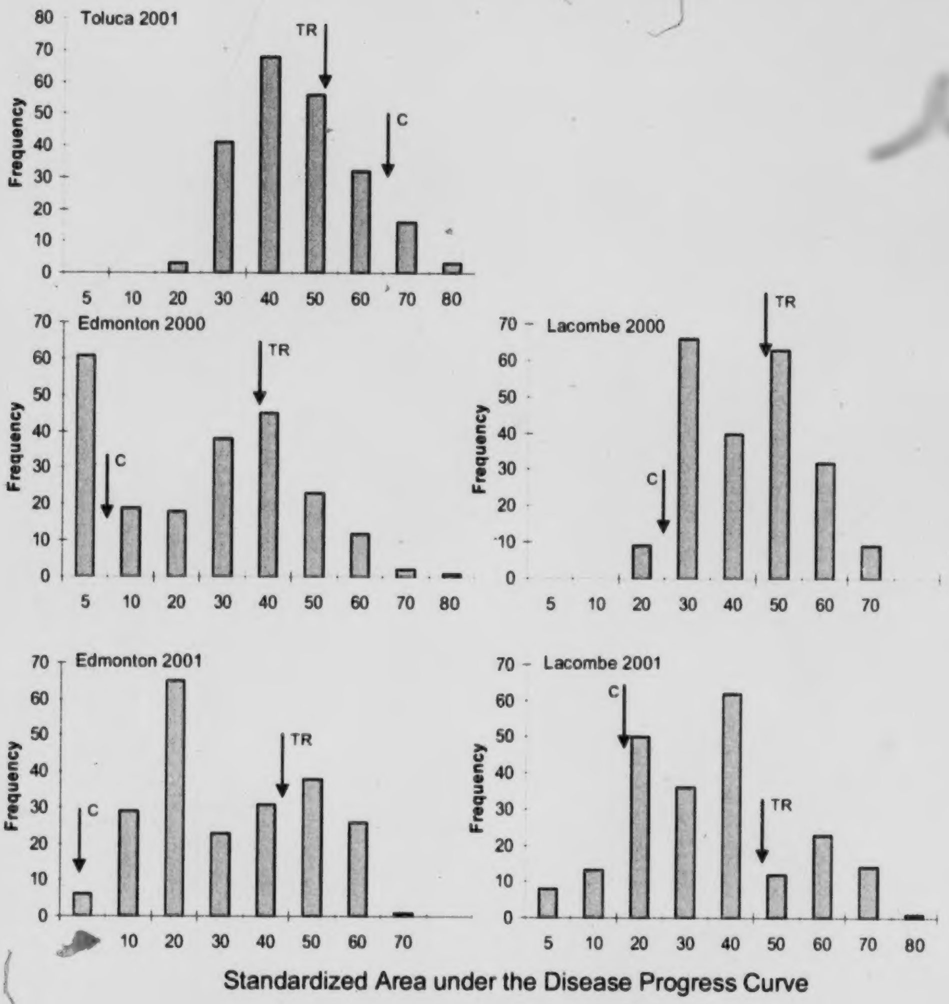

Figure 6-5. Frequency distribution of standardized area under the disease progress curve (SAUDPC) for scald severity of 219 double haploid recombinant inbred lines of the population CDC Dolly and TR 251 studied in Edmonton, Lacombe, Canada, and Toluca, Mexico. Arrows show the SAUDPC means for the parental lines (C: CDC Dolly; TR: TR 251).

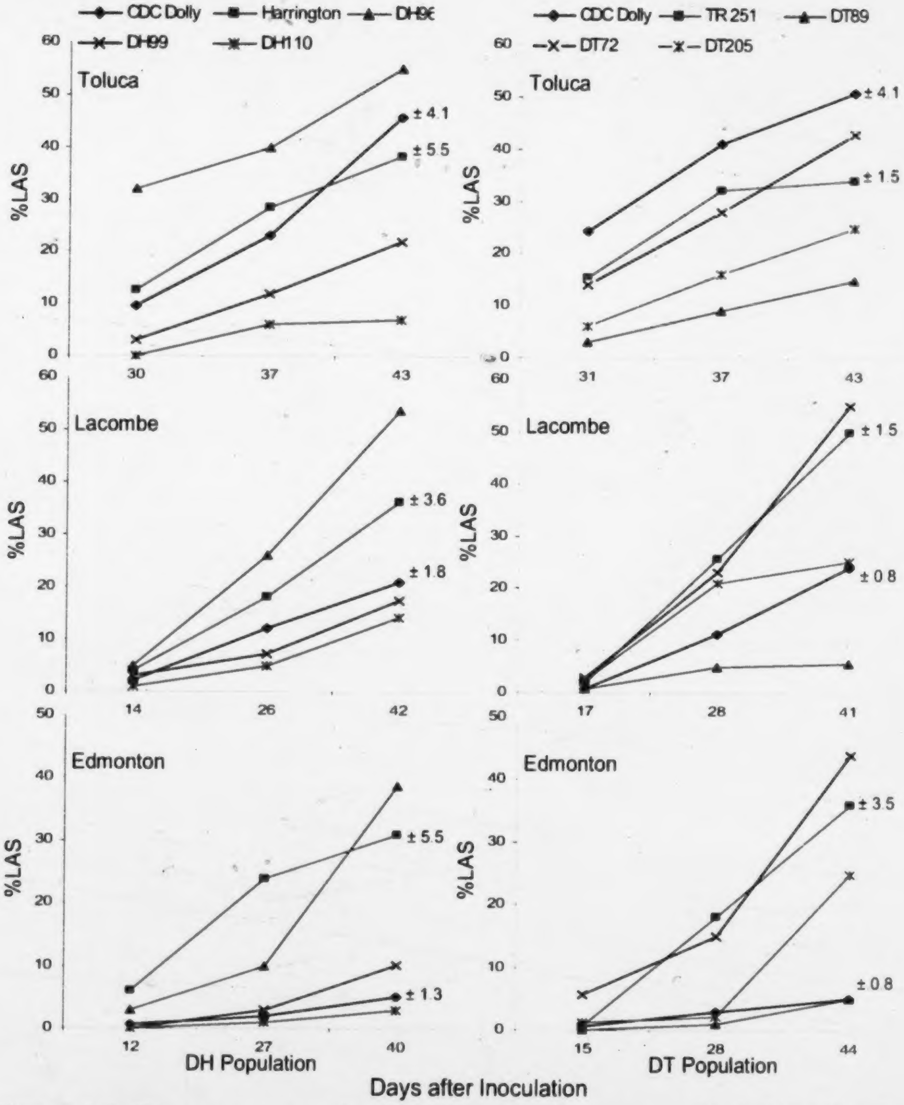

Figure 6-6. Disease progress for the percentage leaf area scalded (%LAS) of a set of three recombinant inbred lines of the populations of CDC Dolly 6 x RFLP Harrington (DH) and CDC Dolly x TR 251 (DT) and their corresponding parents studied in Edmonton, Lacombe, Canada, and Toluca, Mexico. Standard deviations are shown for the parents.

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Chapter 7

Summary, discussion, conclusions, and future perspectives

7.1. Introduction

Both barley and scald are important globally. Canada is among the top producers of barley around the world, and Alberta accounts for a major portion of barley production in this country. Scald of barley caused by *Rhynchosporium secalis* (Oud.) J. J. Davis (syn. *Marssonia secalis* Oudem.) can adversely affect both quality and yield of barley, and has been particularly troublesome in Alberta. The majority of cultivars registered to be used in western Canada are susceptible to scald. Also, cultivar resistance has been reported to be short-lived in several cases. Hence, slow-scalding resistance (S-SR) has been gaining increasing attention in barley breeding programs due to concerns regarding the instability of resistance.

Little has been known about the nature and inheritance of S-SR. Using spring barley genotypes with various levels of resistance against *R. secalis*, a series of studies were undertaken to thoroughly investigate the barley-scald pathosystem with respect to characterization of the slow-scalding phenomenon. More specifically, the specific objectives were to optimize techniques for optimal development and assessment of scald under controlled and field conditions; to determine the presence or absence of S-SR in western Canadian barley cultivars (WCBC) studied in Canada and Mexico; to examine qualitative reactions of many barley cultivars against several Canadian and Mexican isolates of scald, and to test for differential virulence and aggressiveness of the isolates studied; to describe the infection process, elemental changes, and disease/resistance related factors in some resistant, slow-scalding, and susceptible cultivars, and to investigate the effects of the scald disease on transportation of vascular exudates through barley leaf auricles; and to investigate the genetic basis of slow-scalding resistance in genetic populations derived from 3 ICARDA/CIMMYT slow-scalding lines, and one Canadian slow-scalding variety, CDC Dolly.

7.2. Summary and discussions

7.2.1. Techniques for optimal development and assessment of barley scald

Here, the objectives were to optimize indoor and field techniques to achieve an effective differential infection, and to develop an effective rating scale for the assessment of scald at both the seedling and adult plant stages. In order to study S-SR effectively, indoor and field techniques were optimized for a uniform development of infection in barely genotypes, and a precise assessment of scald at both seedling and adult plant stages. Unfavourable conditions such as high temperature and low humidity can alter disease development and progress. In our indoor facilities, key problems were variation caused by the ventilation and humidifying systems used for controlling temperature and humidity. These were addressed by keeping the inoculated plants at 15 – 18°C using a sealed transparent polyethylene cover under high relative humidity (95-100 RH%) for about two weeks. This was followed by a dry period, when the barley seedlings were placed in a polyethylene tent and were sprayed twice a day with Milli-Q® water during the 3- to 4-leaf stage. The dry period and the spray of Milli-Q® water seemed crucial for development of typical lesions and sporulation of the pathogen. A prolonged excess in leaf surface wetness was rationalized to cause the formation of atypical lesions of scald and/or complete wilt of the inoculated leaf tissues. Similar temperature and humidity regimes and tent structures were used in the University of Alberta's walk-in chambers and resulted in development of scald on adult barley plants. This could help plant pathologists and breeders to closely study the reactions of several barley genotypes to a single isolate of the pathogen at adult-plant stage under controlled environments. In this set-up, unlike under field conditions, barley-scald interactions are not influenced by competition among different pathotypes of the pathogen or even competition/antagonism between different pathogens. These conditions would help plant breeders to minimize genotype by environment interactions and get a clear sense of reactions of their genotypes challenged by separate single-spore isolates of the pathogen. Plant pathologist could also benefit from such a standardized facility to identify races of the pathogen and characterize their pathogenicity. It has been stated that uniform selection for horizontal resistance is possible only if plant materials are exposed to a single race of a given pathogen where that race has matching virulence gene(s) for all the R genes conditioning vertical resistance in the plant populations under study (Van der Plank, 1984). Identification of such a race is facilitated once differential plant genotypes could be exposed separately to a wide array of single-spore isolates of the pathogen at both seedling and adult-plant stages. Besides a routine seedling test, indoor adult-plant tests would enable scald workers to run at least three sets of adult-plant evaluations for thousands of barley breeding lines during a year, even during periods when field evaluations are not feasible. Additionally, the feasibility of indoor applications of different spectra of virulent isolates of *R. secalis* rather than only those of local origin would help us to breed for durable resistance, where the import of isolates from other geographical districts is prohibited and/or national/international evaluations problematical.

Under field conditions, the straw inoculum was found to be as effective as spray inoculum in initiating the scald disease. However, with respect to the negative aspects of straw inoculum in spreading other stubble-borne diseases like net blotch and spot blotch, caution must be taken when applying the straw inocula, to avoid problems associated with the presence of other pathogens of barley in a scald nursery. In contrast to their negative aspects, applications of the infested straw inocula of a barley genotype fully susceptible to scald and resistant to other local diseases of barley could be practically easier and economically cheaper than those of the spray inocula.

With respect to developing a scoring scale, a modified 0-9 scale, which integrates different aspects of disease intensity into one single score, was proposed for assessment of adult plant/slow-scalding resistance. As compared to the previously used scales, this scale can be considered similar to those proposed by Couture (1980) and modified by Burnett and Helm (1995). However, scores in this scale can represent the level of scald intensity in each defined resistance class more accurately. This can lead to an effective screening for S-SR where the scale provides breeders with more bench mark for intermediate classes of resistance to scald. Another 0-4 infection response rating scale was proposed for evaluation of seedling resistance. As compared to the previously used scales, this scale is considered similar to those used by Tekauz (1991) in terms of emphasizing on scald infection types rather than focusing on quantitative difference of the disease intensity at the seedling stage. The various infection types and the percentage area scalded were also illustrated in the developed scales. This could help raters to record disease scores in a fast, easy, more precise, and reproducible fashion.

7.2.2. Western Canadian barley cultivar tests

In order to determine the presence or absence of slow-scalding resistance in WCBC, quantitative reactions of 38 western Canadian barley cultivars (WCBC) to mixtures of scald isolates under different field conditions in Canada and Mexico were determined and compared to those of 9 scald differentials, 2 ICARDA/CIMMYT slow-scalding lines and one local resistant check during 1999-2001. Similar to this study, in western Canada, using a 0-9 scoring scale, scald resistance is usually evaluated in nurseries comprised of breeding lines, checks, materials from Alberta Regional Variety Entries, and candidate barley cultivars going through the Prairie Registration Recommending Committee for Grain, Western Cooperative Tests. Scores from two sites, Lacombe and Edmonton, are then supplied to the cooperating breeding programs and are used in assessing which lines to advance and what further crosses to make.

In the present study, areas under the disease progress curve, and apparent infection rates were considered useful to measure whether scalding was fast or slow. Among the barley genotypes studied within each environment, there were slow-scalding genotypes as characterized by their compatible reactions with scald and low to intermediate levels of disease progress at the adult plant stage. However, as it was shown, due to the effects of environments, scald isolates, and genotype x environment interactions, the barleys studied did not necessarily perform in a similar way in different locations/years. This indicated that genes conditioning S-SR could not equally operate against all pathogenic races of *R. secalis*; and, hence might be race-specific. On the other hand, resistance level in ICARDA/CIMMYT slow-scalding lines and some Canadian genotypes to different spectra of the local pathotypes of *R. secalis* applied were showed to be stable. This indicated that non-race-specific resistance was also involved in S-SR. The presence of an adequate level of quantitative (background/non-race specific) resistance coupled with the effects of race-specific resistance were considered likely to govern resistance in the slow-scalding lines studied (e.g. CDC Dolly).

A comparison of disease reactions between a set of differentials, local slow-scalding lines, or resistant checks, and a genotype of interest in different environments could provide scald workers with useful genetic/breeding/disease management information. In this study, reactions of the genotypes were compared with those of the differentials with known major genes for barley scald resistance. Our results indicated that differentials with major genes could show different quantitative performance to scald depending on the environment used to assess their field reactions. In this respect, the resistance in Osiris looked quite stable and promising, as reported by previous workers (Ceoloni, 1980; Salamati and Tronsmo, 1997; Xi *et al.*, 2002). Regardless of the quantitative resistance backgrounds of differentials, the information obtained from such a study could result in monitoring the effectiveness of resistance genes under field conditions in different environments and help barley pathologists to manage scald effectively. For example, it was found that genes conditioning resistance in Atlas and Atlas 46 were only effective in Canada where as those in Osiris were stably effective in the all test sites. Therefore, incorporating resistance from the genotype such as Osiris into the barley breeding materials of the two countries deems essential. Additionally, genes which their resistances are not effective over different locations should only be used in the area in which their resistances have not been overcome.

7.2.3. Pathogenicity tests

These tests were undertaken to examine qualitative reactions of 73 barley genotypes with different resistance /susceptibility levels against a set of 19 single-spore isolates of *R. secalis* from Canada and Mexico at seedling stages, and to test for differential virulence and aggressiveness of the isolates studied. Nineteen different pathotypes were identified out of the 19 isolates studied. These isolates were among a group of 250 single-spore isolates collected for the purpose of this study. The results obtained from this investigation were indicative of a large pathogenic variability around the experimental plots studied in both Canada and Mexico. This was in agreement with the majority of studies published indicating extreme pathogenic variability of *R. secalis* in different parts of the world (Goodwin *et al.*, 1993; Jörgensen and Smedegaard-Petersen, 1995; McDermott *et al.*, 1989; Tekauz, 1991). However, neither pathogenicity of the Mexican isolates of *R. secalis,* nor the reactions of such a set of barley genotypes have been studied so far.

In this study, none of the isolates was virulent against all the barley genotypes. However, referring to the reactions of differentials on the pathotypes identified, it was inferred that a complex array of different alleles/genes was likely involved in the pathogenicity of isolates studied. Based on their reactions to all isolates, plant materials were also classified into different clusters representing various resistance levels. The results showed that none of the barley genotypes were immune to all the isolates whereas there were some malting, hulless, slow-scalding, and differential lines resistant to the majority of pathotypes. Since the isolates studied were pathogenically different and represented high levels of aggressiveness, it was rationalized that resistance to such a complex array of virulent pathotypes could be promising as a potential source of stable resistance to scald. This study also revealed that the Canadian isolates were less aggressive than those originating from Mexico. It was also shown that most Mexican isolates studied were highly virulent on the "resistant" Canadian barley cultivars. These results were in accordance with our findings that the Canadian sources of resistance e.g. Seebe, Mahigan, and Kasota were found to be fully susceptible in field conditions in Mexico.

Therefore, it was rationalized that the effectiveness of resistance factors in each genotype depended on the pathogenicity of local populations of scald in a given geographic area. Consequently, it is suggested that any seed import into Canada from Mexico must be highly controlled to avoid a possible break down of resistance genes which are still effective in Canada. In contrast, in order to incorporate more resistance factors into the breeding lines of both Canadian and ICARDA/CIMMYT barley programs, introduction of resistance resources from one location to the other is strongly recommended. As it was observed, many ICARDA/CIMMYT resistant and slow-scalding genotypes could be used as ideal sources of scald resistance in Canadian barley breeding programs whereas cultivars such as AC Harper and AC Hawkeye could also enter into the crossing programs at CIMMYT. Since ICARDA and CIMMYT mandate international collaborative barley breeding programs throughout the world, their crossing programs have involved incorporating many resistance genes into barely breeding lines. Hence, the genes conditioning resistance in the ICARDA/CIMMYT barley genotypes including those used in this study are likely a core set of effective resistance genes from all different parts.

7.2.4. Histopathological and physiological studies

In these studies, with an emphasis on the characterization of S-SR, the objectives were to describe the infection process, elemental changes, and disease/resistance related factors in resistant, slow-scalding, and susceptible cultivars, and to investigate the effects of different severities of disease on transportation of vascular exudates through barley leaf auricles. To pursue these objectives, a set of 5 genotypes, Osiris (resistant), Zavila and UNA 80 (slow-scalding), and Stander and Jackson (susceptible) were studied using different techniques and also exudate collection and analysis techniques in conjunction with protein and sugar assays.

In the histopathological studies published so far, resistant and susceptible reactions to scald have been well addressed. The present study was undertaken to characterize infection process and elemental changes in lines with S-SR as compared to those of susceptible and resistant genotypes. In this investigation, in contrast to the resistant check, the infection process in slow-scalding lines found to be similar to that of susceptible cultivars. However, the results revealed that in the slow-scalding cultivars, the rates of sporulation were lower compared to those in the susceptible genotypes whereas no sporulation was observed on the resistant cultivar. As compared with susceptible checks, slow-scalding genotypes also showed longer latent periods, less frequent and smaller lesions, and resistance against secondary infections. These characteristics could reduce disease severity and progress and play an important role in slow-scalding type of resistance. The formation of Ca/K containing crystals was found to be associated with disease at all resistance levels. This was in agreement with the results obtained by Tewari (2000). Significant differences were observed in the studied elements between inoculated and uninoculated samples implying the involvement of active mobilization and/or sequestration of elements in this pathosystem. The cultivar Osiris showed a significantly different elemental composition, whereas no particular differences were evident between slow-scalding and susceptible lines.

Changes in the host vascular bundles and transportation of vascular sap under different disease severities were studied by light and transmission electron microscopy, and also by analysis of exudates in conjunction with protein and sugar assays. It was found that only in severe auricle infections, phloem cells were macerated and transportation of photosynthates from the leaf into the plant was adversely affected. This would be the first report on the effect of scald infection on transportation of materials through the auricle.

7.2.5. Genetics of slow-scalding resistance in spring barley

The genetic basis of slow-scalding resistance was investigated in several Canadian and ICARDA/CIMMYT genetic populations. The susceptible parents (Stander, TR 251, and Harrington) showed a high level of disease progress as determined by standardized area under the disease progress curve (SAUDPC). The ICARDA/ CIMMYT slow-scalding parents (UNA 80, Zavila, and CI1240/FOMA//CI6239.15D) showed low to intermediate levels of resistance to both Canadian and Mexican isolates of *R. secalis* whereas CDC Dolly reacted as a slow-scalding line only in Canada. It was shown that the quantitative reactions of slow-scalding genotypes may vary depending on the disease pressure and the pathogenicity of the mixture of scald isolates applied indicating the significance of environment and genotype x environment interaction effects. Variability in disease reaction could be mainly related to differences among the array of virulence genes involved in the pathogenicity of the applied inocula in each environment. Continuous variation was observed among all the populations studied. Interestingly, distributions of RILs in the populations of CDC Dolly at Toluca where both parents were susceptible, were also continuous consistent with the concept that the inheritance of S-SR was quantitative. Transgressive segregation was observed among the progenies of most crosses indicating that the parents should have different genes for S-SR. Even Harrington, TR 251, and Stander as susceptible parents were also found to carry at least one resistance factor for S-SR. Resistance to scald in such susceptible genotypes was most likely not of a major-gene type. Hence, it was inferred that at least one minor gene conferred background resistance in these genotypes. Additive variance was found to contribute the major portion of total genetic variance for S-SR. Non-additive portion of genetic variance was found to be statistically significant indicating that in addition to additive effects, dominance and epistatic effects were likely contributing to S-SR. Since both additive and non-additive effects are sensitive due to the effects of environment and GxE effects, the possibilities of making errors in over- or under-estimating the genetic effects may not be ignored. Additionally, errors due to visual assessment of disease severity and failure to meet diallel analysis hypotheses might cause difficulties in the analyses and interpretation of genetic studies. The results also revealed the existence of 1-4 resistance genes with additive effects and high heritabilities in the slow-scalding parental lines. It was concluded that dealing with such resistance genes with additive effects would facilitate breeding for more stable resistant genotypes to scald. Among the studies carried on to investigate the inheritance of resistance to scald, there are only few publications which refer to quantitative inheritance of resistance to scald (Kari and Griffiths, 1993; 1997). The present study is the first report on the inheritance of S-SR. More studies using other ICARDA/CIMMYT slow-scalding barley genotypes are needed in order to achieve a better understanding of the inheritance of slow-scalding and identifying more genes for slow-scalding.

7.3. Conclusions

A thorough study on a given plant-disease pathosystem requires investigations over all the factors of the disease pyramid, the disease causal agent, the host, environment, and time. A dynamic research on the pathosystem for a proper management of the disease, on the other hand, requires information about all aspects of qualitative and quantitative interactions between the host and the pathogen at the seedling and adult-plant stages. In the present thesis, using thousands of plant genotypes and many fungal isolates, the barley-scald pathosystem was studied in different environments during 1999-2003 to elucidate various aspects of slow-scalding.

Techniques and scoring guides leading to the alleviation of problems involved in optimal development and effective assessment of *R. secalis* at the University of Alberta indoor facility were developed (Chapter 2). These studies helped achieve some degree of standardization in the disease assessment methods. The results from this study confirmed that the indoor conditions were conducive to highly repeatable results, and thus reliable for evaluating scald at both the seedling and adult plant stages. These techniques and guidelines were further effectively used to conduct routine scald seedling and adult-plant tests outlined in Chapters 3 to 6. To the best of our knowledge, this would be the first report on successfully carrying out both tests together under controlled environments.

Additionally, so far, there has been no attempt to develop illustrated scoring guides for both seedling and adult plant assessments of the barley scald.

The reactions of many Canadian barley cultivars to mixtures of scald isolates were assessed and their adult-plant resistance levels were quantified under different field conditions in Canada and Mexico. Slow-scalding genotypes were identified by the presence of susceptible reactions at the seedling stage and adequate levels of field resistance at the adult plant stage. The results obtained could provide both barley breeders and pathologists with an insight into the quantitative reactions of Canadian lines/cultivars across different test environments. Since race-specific resistance was also considered to contribute to S-SR, as a practical solution, it was suggested that instead of aiming at the durability introduced by Johnson (1993), stability over locations/years be used to develop effective resistance against the local spectrum of isolates of the pathogen and manage their effectiveness by a gene deployment/rotation strategy. Deployment of effective and more stable resistance slow-scalding genes while placing less selection pressure on the pathogen populations, would assist breeders/pathologists in managing the scald disease in a more sustainable way.

In the pathogenicity tests (Chapter 4), qualitative reactions of several barley cultivars/lines against various single isolates of *R. secalis* originating from Canada and Mexico were studied both to examine the virulence and aggressiveness of the isolates, and to determine the potential stability/durability of resistance to scald. Neither the pathogenic variation in isolates from the "scald hot spot" of Toluca, Mexico, nor the reactions of this set of barley cultivars/lines against the Canadian and Mexican isolates have been previously tested. In the WCBCT study presented in Chapter 3, a sub-set of barley genotypes studied were artificially inoculated with mixtures of different local isolates of the pathogen and field evaluated at the adult-plant stage in Canada and Mexico. The pathogenicity study enabled simultaneous examination of the reactions of many barley genotypes to several *R. secalis* isolates originating from both countries separately at the seedling stage. Since Canada and Mexico are collaborating and aiming at durable resistance for barley diseases, the information obtained from such studies can assist barley pathologists to identify arrays of pathotypes representing pathogenic variability of the scald populations at target breeding locations. Additionally, barley breeders could select among materials tested against isolates representative of both countries in a controlled environment and hence could breed for more effective resistance against the disease without environmental release of alien races of the pathogen. Including a set of differentials consisting of isogenic lines differing only in all scald major-genes can be beneficial in monitoring the effectiveness of resistance in each target environment. Results indicated that resistance in Osiris, as compared to the other differentials studied, could be considered as a promising potential source of stability to scald. It is suggested that a core set of promising barley genotypes studied in the present work should be examined against all the scald isolates belonging to Dr. J.P. Tewari's barley scald culture collection. This would result in establishing a core set of isolates with differential responses to available resistance factors.

The results from a histopathological study on a set of susceptible, slow-scalding, and resistant barley cultivars are presented in Chapter 5. In this chapter with an emphasis on the characterization of S-SR, infection processes and elemental changes in the epidermal cells in the barley genotypes studied were compared before and after inoculation with some isolates of *R. secalis*. Histopathological differences existed amongst the genotypes. In summary, slow-scalding lines were capable of reducing disease severity and suppressing the disease progress by preventing large coalescing lesions, by having a longer latent period, and by having lower sporulation rates. It was implied that the host might cause/trigger the production of a self-inhibitor by the fungus which could serve to suppress the enlargement of a lesion, or the initiation of next cycle of the disease. Further research could contribute to the knowledge of expression of genes involved in recognition of avirulent gene products of the pathogen, signal transduction, and induced resistance response to infection. A better understanding of host resistance and fungus pathogenicity mechanisms would help barley breeders and geneticists to enhance the genetic gain in resistance breeding methodologies. For example, application of molecular techniques can effectively help scientists in identifying, screening breeding materials, and pyramiding of resistance factors (using both major genes and QTLs). The results from a study on changes in the host vascular bundles and transportation of vascular sap under different disease severities are also presented in Chapter 5. These results could have significant implications in the proposed scoring scale in Chapter 2.

Microscopical study on healthy vs. infected auricles coupled with protein and sugar assays revealed that severe auricle infections may adversely affect transport of photosynthates from the leaf into the plant. Hence, a score representing a high level of susceptibility is better to be assigned to plants with high rates of severe auricle infections, even if the scalded area only accounts for 5-10 percent of the total leaf blade. This conclusion pays homage to the insight of late Prof. W. A. Skoropad on effects of auricle infection.

Finally, as presented in Chapter 6, in order to determine the inheritance of genes conditioning S-SR, the genetic basis of S-SR was investigated in various genetic populations of crosses between slow-scalding parents originating from Canadian and international barley breeding programs and susceptible genotypes with desirable malting quality. Little was known about the inheritance of S-SR. From the results obtained from the genetic study, it could be deduced that a complex set of additive, dominance, and epistatic gene actions was likely conditioning resistance in the slow-scalding genotypes studied. However, with respect to the high level of heritability and great importance of additive effects in early to advanced generations, it was rationalized that the resistance factors could be readily incorporated into barley breeding materials. It was discussed earlier that using phenotypic selection for S-SR in early generations, and simple or convergent back-crossing methodology, genotypes with S-SR can be identified, and target genotypes can be enriched with resistance gene(s), respectively. To achieve stable resistance against barley scald, it is suggested that following the identification of genes resistant to representative pathotypes of a target locations, they are pyramided into a given genotype with an acceptable level of quality and agronomic characters. McDonald *et al.* (1988) discussed that pyramiding different resistance genes together into a single cultivar could be one possible way to increase the durability of resistance to scald. However, two issues may arise; identifying and introgressing different resistance genes into a target genotype, and considering a yield penalty due to combining the resistance genes in a given high yielding and agronomically adapted cultivar. In this study, in order to facilitate achieving malting barley genotypes with stable resistance to scald, Harrington, Stander, and TR 251 with desirable malting features were crossed to a set of slow-scalding genotypes where various kinds of recombinations were expected to occur among genes governing resistance and quality traits. Derivatives from this study may be useful for the improvement of scald resistance in malting barleys in western Canada. As several RILs with S-SR have been identified in this study, a complementary quality study on such genotypes is likely to assist breeders in identifying and introducing malting barley varieties with stable resistance to S-SR. No such a malting barley has yet been identified/registered/used in western Canada.

7.4. Future perspectives

7.4.1. Scald management

It is important to manage scald in Canada and other regions of the world where *R. secalis* finds its favourable habitat to grow. In Alberta, the average yield losses due to scald have been as high as 5-10% depending on the year which is equal to monetary losses of over $30 million (Turkington *et al.*, 1998). The scald of barley can be managed using different approaches including genetic resistance, cultural, and chemical control measures. However, as in many other diseases of crops, none of the available solutions may result in long-term and effective management of the disease alone. For example, the application of resistant cultivars in Canada has always been considered an effective and environmentally-friendly method of controlling disease, whereas the resistances of genes incorporated into the registered cultivars have been broken down repeatedly (Xi *et al.*, 2002; Xi *et al.*, 2003). The tremendous selection pressure placed on the scald pathogen due to extensive use of the same resistance gene coupled with the extreme pathogenic variability of the scald pathogen has been attributed to cause new races (with new virulence and/or more aggressiveness) to evolve and/or to increase the prevalence of existing virulent pathotypes to significant levels. An integrated management of scald which could reduce the disease pressure and severity, not leading to the formation of new pathogenic races of *R. secalis* is likely to be the most effective way of preventing economic losses in the crop due to the disease. As suggested by Turkington *et al.* (1998), slow-scalding is considered as a stable resistance to control selection pressures on the scald pathogen in response to changes in the resistance-virulence spectrum and to reduce yield losses due to *R. secalis*. Since the stability of this kind of resistance is relative to the pathogenicity of the pathogen population, a continuous monitoring of the pathogen in target environments coupled with testing of the effectiveness of host resistance genes are two prerequisites for systematic disease management. It was discussed earlier that studies on the reactions of barley genotypes to local, regional, and international sets of scald pathotypes assist barley breeders and pathologists in resistance gene deployment. Following a gene rotation methodology, a gene whose resistance has been broken should not be used in areas where its corresponding virulent gene is prevalent, whereas it can be deployed in the regions where its matching race(s) does not occur. On the other hand, breeders should not use all effective genes simultaneously at one target location where/when they are not needed. This will further lead to availability of alternative sources of resistance and quick access to the resistance gene resources in the case of sudden outbreak of new virulent genes. This methodology will be successful if the advanced lines of crosses between desirable adaptive barleys and parents conferring resistance to the pathogen are available well in advance of the disease outbreak. Recurrent selection methodology can also be a useful tool to increase the frequencies of favourable agronomic and resistance alleles in the genetic populations. Lines drawn from such populations could also serve as ideal genotype in barley crossing programs.

In "hot spot" disease nurseries like Toluca, where resistant plant genotypes have been long exposed to environmental conditions conducive to disease development, the number of virulence genes is expected to be higher than other places where the pressure of disease is less. Since the scald pathogen is seed borne, care must be taken not to import/export any contaminated seed, as it can bring new sources of pathogen virulence into the target country. This study has shown that virulence/aggressiveness spectra of the pathogen in Mexico are different than in Canada. Mexico is an international source of breeding and broadcasting seeds and supply of infected seed from there may result in breaking down of available resistance sources in our regions in Canada. Once it happens, breeders/pathologist will have to devote tremendous efforts to fix the problem. Therefore, it seems much easier to manage the problem with basic quarantine practices. Transportation of seed and other sources of primary inoculum from Lacombe to other experimental sites in Canada can also be considered to be a similar risk.

The application of biological measures to control barley scald has so far not received considerable attention. However, information on bioelicitation of host defense response to the scald disease may lead to development of novel environmentally-friendly scald disease management strategies. Bioelicitors are thought to be the next generation of crop protectants and worthy of further investigation with respect to the scald disease (Tewari, 2000).

The applications of cultural control measures, proper agronomic practices for the preparation of soils before planting and after harvesting, and rotation with non-host crops must also be jointly considered important in the management of scald. Continuous cultivation of barley and use of minimum tillage practices can lead to evolution of new races of the pathogen, and breaking of the resistance genes in a shorter time. Chemical control, despite its effectiveness, should be considered as the last solution since that is not an environmentally-friendly approach. However, when other measures might not reduce the yield and quality losses to an acceptable economic level, the application of fungicide like Tilt® can assist the barley grower to minimize the adverse effects of disease in their fields. Application of fungicides may also result in emergence of fungicide resistant races of the pathogen. In an improved integrated *R. secalis* management system, emphasis should be placed on ongoing investigations of all possible measures of controlling the disease where chemical control strategies must apply more efficient and environmentally safe molecules. A better understanding of proteoms involved in pathogenicity and resistance (PR proteins) would play an important role in scald disease management. It also may result in safer and faster approaches towards developing/applying efficient fungicides for controlling more aggressive races of the pathogen.

7.4.2. Durable disease resistance

At CIMMYT the key success in breeding genotypes with resistance close to immunity and a high level of stability in the wheat-rust pathosystem, has been achieved by pyramiding 3 to 4 minor genes for slow-rusting into their wheat breeding lines using a simple back-crossing approach (Dr. R. P. Singh, CIMMYT Wheat program, Mexico, personal communication). For S-SR, similar results would be expected upon pyramiding minor additive genes in a target genotype. To monitor and select for genotypes with a higher number of resistance factors MAS could efficiently help breeders to screen their materials by overcoming GxE interactions. The molecular marker studies on scald resistance have helped barley breeders and pathologists to identify and study genes with major effects (Abbott *et al.*, 1992; Abbott *et al.*, 1995; Barua *et al.*, 1993; Graner, 1996; Graner and Tekauz, 1996; Patil *et al.*, 2003) and QTLs associated with resistance (Backes *et al.*, 1995; Jensen *et al.*, 2002; Sayed *et al.*, 2004). Selected lines will still require validation of resistance in field trials.

During the present study, scald resistance gene were sought in the population of RFLP Harrington X CDC Dolly 6 using the kit AFLP System I (consisting of EcoR I and Mse I primer combinations). This system in conjugation with bulk segregant analysis showed some polymorphic bands corresponding to susceptible and resistant bulk RILs'DNA in both pulse and repulsion arrays. However, none of studied polymorphic bands were statistically associated with the resistance/susceptibility in the population (data are not presented in this thesis). Further search for linked markers is desirable to support breeding of pyramided genes.

7.4.3. Multiple disease resistance

In Alberta, as in many other barley growing areas, scald and net blotch are major production constraints. Fusarium head blight and spot blotch are becoming more widespread and may become serious problems in the region (Clear and Patrick, 2004). Resistance to smut diseases, BYDV, and some other diseases must also be considered in the future. As it was shown in this thesis, among studied ICARDA/CIMMYT genotypes there were some sources of resistance to more than one disease. Therefore, it seems advisable to simultaneously incorporate these sources of multiple resistance into the target breeding lines. Breeding for multiple disease resistance in the test locations where the weather favors the growth of more than one common disease of barley is practically feasible. Saghai Maroof *et al.* (1983) presented a good example of breeding multiple resistance to three diseases of barley, scald, net blotch, and powdery mildew, which was build up into the progenies of advanced generations of barley Composite cross II. Breeding for multiple disease resistance is one of the main objectives of the ICARDA/CIMMYT barley breeding program (Capettini *et al.*, 2002) where multiple resistance to five diseases including BYDV, stripe rust, leaf rust, scald, and stem rust have been incorporated into agronomically improved genetic backgrounds of both six-

and two- row genotypes.

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Appendix I: Supplementary literature review

Artificial inoculation

In addition to the spray method, different inoculation techniques have been used for indoor experiments including placing a droplet of spore suspension in the small natural funnel formed by the emerging leaf (Evans and Griffiths, 1971), inoculating detached leaf segments with a droplet of spore suspension (Williams and Owen, 1973), and placing a small piece of scald culture grown on LBA onto barley leaves spore side down (Habgood, 1977). The spray method has been favored by the most workers. It has been considered effective, is easy to use, and is readily applicable for large scale tests. The inoculations for seedling tests have been reported to be sprayed at ZGS 12 when the second leaf is unfolded (Ali and Boyd, 1973; May and Harper, 1989; Saghai Maroof *et al.*, 1983), at ZGS 13 (Salamati and Magnus, 1997; Xi *et al.*, 2002; Xue and Hall, 1991), at ZGS 14 (Rotem *et al.*, 1976), or at ZGS 15 (Xue and Burnett, 1995). Additionally, the concentration of sprayed inoculum has been different among the tests. For example, 4 x 10^3 (Xue and Hall, 1991), 5 x 10^3 (Xue and Burnett, 1995), 3 x 10^5 (May and Harper, 1989), 1 X 10^5 (Rotem *et al.*, 1976; Salamati and Magnus, 1997), and 2 x 10^5 spores mL^{-1} (Ali and Boyd, 1973; Saghai Maroof *et al.*, 1983) have been applied to different number of plants/pot. Schein (1958) found few if any discernible differences among symptoms on plants inoculated with a series of spore concentrations over 5 x 10^4.

Other diseases of barley and their interactions with scald

Environmental conditions under which the scald experiments have been carried out have in many cases been shown to be suitable for the development of other diseases (Burnett and Helm, 1995; Yitbarek *et al.*, 1998) that may interact with *R. secalis* in a number of different ways (Jenkyn *et al.*, 1989; Riddle and Briggs, 1950; Xue *et al.*, 1994). Under such circumstances, the scald-infested straw may harbor other pathogens of barley as well. Riddle and Briggs (1950) stated that the presence of net blotch (caused by *Pyrenophora teres* Drechs.) had made their field evaluation of scald difficult and uncertain. Jenkyn *et al.* (1989) studied the effects of straw inoculum and fungicide on *R.*

secalis growth and observed that powdery mildew caused by *Erysiphe graminis* DC. F. sp. *hordei* Em. Marchal was commonly least severe in plots with most scald infection. They concluded that severe scald had lessened the amount of susceptible tissue available for infection by powdery mildew. Intensive surveys in Alberta have shown that scald and net blotch are widespread in the region (Burnett and Helm, 1995). Xue and Burnett (1995) observed the predominance of *P. teres* over R. *secalis* even when inoculation with the scald pathogen preceded the inoculation with the net blotch pathogen. They also reported the antagonistic effect of net blotch on scald at the seedling stage under specific inoculum concentration, wetting period, and incubation temperature. Xue and Burnett (1995), on the other hand found no antagonism between scald and net blotch under field conditions. Despite the fact that most Canadian barleys are susceptible to both diseases and that the net blotch pathogen is wind borne with a shorter disease cycle, scald severity is higher than net blotch in most studied locations in Alberta (Burnett and Helm, 1995).

Disease assessment

Diseases of a crop are assessed to provide plant breeders/ pathologists/ geneticists with data on the plant resistances (seedling and/or adult plant) or their components to the corresponding pathogens, the pathogenicity of the causal agents (virulence and/or aggressiveness), and related yield losses (Kari and Griffiths, 1997; Khan and D'Antuono, 1985; Salamati and Tronsmo, 1997; Webster *et al.*, 1980; Xi *et al.*, 2000; Xue and Hall, 1991). With the exception of components of resistance, which are only determined by measuring or counting, other aspects of disease can be also assessed by rating/scoring of their type of reaction (the degree of compatibility), intensity (severity and incidence), and development i.e. rate and amount of infection over the course of epidemics (Kranz, 1988). The type of reaction to a given disease can be easily determined a few weeks after planting at the seedling stage while disease intensity is assessed to differentiate among different levels of resistance or susceptibility at the adult plant stage. The rate and degree of disease progress are also driven from the data obtained from the field response of the adult plants during the course of epidemics (Campbell and Madden, 1990). The phenomenon of slow disease development (e.g. slow-rusting and slow-scalding) is studied using such data (Ginkel and Vivar, 1986; Johnson and Wilcoxson, 1979).

Disease measurement should always be related to defined growth stages of the crop or according to the number of days after sowing/emergence (James, 1971). Accordingly, the disease evaluation date has to be an appropriate time after inoculation and at a known growth stage of the crop. Zadoks' growth stages of small grains (Zadoks *et al.*, 1974) are applicable to barley except for the flowering stages (ZGS 61-69), which overlaps with the ear emergence's (ZGS 51-59). To avoid complexity, using only ear emergence stages will be better.

Scald seedling evaluation scales

As shown in Table A-1, various rating scales have been used to assess the reaction of seedlings to *R. secalis* (Ali and Boyd, 1973; Ceoloni, 1980; Jackson and Webster, 1976; Jörgensen *et al.*, 1992; Sarasola and Campi, 1947; Schein, 1958). A scale with five classes (0-4) was used to classify the reactions of several barley scald varieties and their crosses' progenies in F2 and F3 segregating populations both under greenhouse and field conditions (Riddle and Briggs, 1950). In such undefined scales, distinction between resistant or susceptible reactions were not clear. Schein (1958) modified a 5-part lesion type scale of Sarasola and Campi (1947) into a descriptive 0-3 scale, which was a combination of Sarasola and Campi's scale with an additional lesion description for each class. The Schein's scale was designated for seedling evaluation based on scald infection type. Another descriptive scale of 0-4 similar to that of Schein (1958) was used to classify the reaction types of hundreds of barley varieties/lines against *R. secalis*, where in addition to coalescing large lesions and wilted leaves, the infection on the first leaf was also considered as a diagnostic characteristic of infection types 3 and 4 (Dyck and Schaller, 1961; Starling *et al.*, 1971). Ali and Boyd (1973) modified the scale introduced by Schein (1958) into a 0-4 seedling reaction type and evaluated the type of reaction of different host species of some genera in response to a mixture of several isolates of *R. secalis* at field conditions and to four separate isolates at seedling stage. The modified 0-4 rating scale has been used by numerous workers to assess reactions of seedlings to a variety of different scald isolates in controlled environment (Abbott *et al.*, 1992; Abbott *et al.*, 1991; Brown, 1990). The Schein's scoring scale was also modified into a four-point scale of 0-3, where lesion types of 0 and 1 were considered as incompatible reactions (Tekauz, 1991). In contrast to those of Schein (1958) and Ali and Boyd (1973), this scale provided the rater with a clear definition of lesion morphology and cut off point for the screening of resistant lines. For example, seedlings with a score of 1 were considered resistant and expected to show very small lesions (up to 3 mm) usually elongated along the leaf margins or being round to oval. However, in this scale, there was no indication of presence of associated dark brown spots with resistance reactions and those lesions at the leaf margins. Additionally, the proposed reaction types was not illustrated to clarify the terminology used. Similar difficulties may be experienced when using the scale described by Jackson and Webster (1976) for seedling tests, where the ratings of 0-2 are indicative of resistant reactions and those of 3-4 are supposed to show susceptible reactions. Similar and/or identical scales were used by other scald workers also to differentiate between susceptible and resistant reactions (Ceoloni, 1980; Garvin *et al.*, 1997; Muona *et al.*, 1982; Robbertse *et al.*, 2000; Saghai Maroof *et al.*, 1983). Using a 0-4 infection type scale, reactions of 18,000 entries from the USDA world barley collection were assessed at the adult plant stage (Webster *et al.*, 1980).

Seedling resistance to a given pathogen must represent disease reaction (infection) type that is compatibility of host to the pathogen. Some scald workers have used a scale of 0-5, using which disease intensities have been measured instead of assessing the disease reaction type at the seedling stage (Bjornstad *et al.*, 2002; Gronnerod *et al.*, 2002; Jörgensen *et al.*, 1992; Patil *et al.*, 2002; Reitan *et al.*, 2002; Salamati and Tronsmo, 1997), wherein the scores of 2-5 could only differentiate among the levels of susceptible reactions at seedling stage and may not necessarily correspond to reactions of the host at adult plant stages (Van der Plank, 1984; Xue *et al.*, 1995). Additionally, according to the 0-5 scale of Jorgensen (1992), disease ratings of 0-2, 2.1-3.0, 3.1-4 and 4.1-5.0 are considered to reflect resistance, moderately resistant, moderately susceptible and susceptible reactions, respectively (Dr. A. Bjornstad, personal communication). How could one consider this scale as a reaction type assessment scale, wherein plants with the scores of 3 and 4 which represent leaves with up to 40 and 80% of scalded areas, are classified as moderately resistant and moderately susceptible, respectively? In another study, the limit for screening resistant reaction was set at LAS < 10 where LAS ≥ 10 to <30 and ≥ 30 were considered moderately resistant and susceptible, respectively (Xue *et al.*, 1995). In addition to the aforesaid 0-5 scale, similar difficulty may be encountered while using its modified 0-3 scoring scale where any score greater than 1.4 is considered susceptible (Jörgensen and Smedegaard-Petersen, 1995; Xi *et al.*, 2002). In summary, disease reaction type must be used for the classification of major gene resistance at the seedling stage (Dyck and Schaller, 1961; Habgood and Hayes, 1971; Starling *et al.*, 1971). In practice, there should be only two descriptive classes (avirulence and virulence) of the pathogen for each of the known host major gene. An avirulence gene in the pathogen can cause compatible reaction with a host lacking its corresponding resistance gene, whereas combinations of resistance genes (Rh/Rrs and rh/rrs) in barley and their corresponding avirulence genes in the fungus result in resistance or incompatible reactions. In contrast, a virulence gene can cause the susceptible reaction even in the presence of a corresponding resistance gene. On the other hand, if quantification of resistance is intended, adult-plant data will provide a more accurate information (Van der Plank, 1984). Care must be taken to distinguish between the qualitative reactions of barley genotypes at the seedling stage to *R. secalis* (infection type or compatibility) and their quantitative field response at the adult-plant stage.

Although, hypersensitive response (HR) has not been reported in the barley-scald pathosystem, there is evidence that could support its presence in conjunction with defense mechanism in the host. Reactive oxygen species (ROS) was reported to play a possible role in the induction of cell death in the susceptible plants during challenge with *R. secalis* (Able, 2003). Interestingly, in the barley-scald interaction ROS, superoxide ($HO_2^{\cdot}/O_2^{-}$) was also found in resistance responses at significant levels (Able, 2003). In other pathosystems, ROS has been reported to play a major role in the initiation of HR, which is a localized mechanism of resistance against obligate parasites and also to signal to other cells in the plant, inducing systematic acquired resistance, SAR (Heath, 2000). Since the scald fungus is a necrotroph and does not need to uptake its required nutrients from living host cells as it lacks a haustorium, HR may not be involved in the resistance response to the pathogen. However, It has been hypothesized that another defense response from the mesophyll cells may be triggered upon a secondary signal generated from the primary interaction of the host and pathogen. This response is the plant cell death due to the avirulence gene products e.g. NIP1 (Steiner-Lange *et al.*, 2003). Hence, the HR-like spots observed in the barley-scald pathosystem could be associated with resistance and be considered as a symptomatic infection type in seedling tests (Dr. A. Bjornstad, personal communication).

Scald adult-plant evaluation scales

Descriptive, numerical, and percentage scales have been frequently used to assess the reaction of barley genotypes to *R. secalis* at the adult plant stage. A descriptive system is defined to provide basic information about the host-pathogen interaction. Descriptive classes have been used to represents reactions of barley genotypes to *R. secalis* at field conditions. For example, a system with only three qualitative levels (resistance, intermediate and susceptible) has been used to extend information from western Canada barley breeding/pathology programs to the hands of barley growers as well as other scientists (Anonymous, 1989-2004). At CIMMYT, a descriptive rating system with more classes (R, TR, MR, MS, S and VS) is used to screen the breeding materials resistant to *R. secalis*, and to determine the sources of resistance at field conditions (Dr. F. Capettini, personal communication). A particular problem appears when one wants to translate these terms into numbers and do statistical analyses over the corresponding data. Besides, there is not a clear definition of each class in terms of its level of disease severity, incidence and progress. Descriptive scores are of little use to scald worker unless they provide breeders/pathologist with an effective means for screening. In South Australia, farmers are told that practically, MR means you may see the disease but will not lose any yield whereas in the VS reaction (score of 9) you may lose up 50% (Dr. H. Wallwork, personal communication).

Various numerical scales have been used to evaluate the reaction of adult plants to the scald pathogen among which 0-9 and 1-9 have been used more frequently. The 0-9 scale was adopted from a scoring system originally developed for the Helminthosporium blight of maize and modified into a scale for appraising the foliar intensity of wheat diseases (Saari and Prescott, 1975). This scale could only provide very basic information about the disease intensity, where for example 0, 5 and 9 corresponds to the presence of disease symptoms on the lowest leaves, the mid-point of the plant, and all leaves and spikes, respectively. The scale was then modified in order to add more details to that introduced by Saari and Prescott (Couture, 1980). As shown in Table A-2A, severity of the disease for each score was defined at various leaf levels. This scale was intended to score the relative disease severity and to give a fair assessment of each plant genotype based on visually evaluated percentage area diseased at each canopy level. However, neither disease incidence nor a clear definition of canopy levels was taken into account. This scoring system with small modifications has been repeatedly used for assessment of scald on national cooperative tests at the University of Alberta and by many scald workers in Canada (Burnett and Helm, 1995; Hall and Xue, 1989; Turkington *et al.*, 1998; Xi *et al.*, 2003). Burnett and Helm (1995) used similar 0-9 scoring system to screen thousands of barley lines in a fast and reliable fashion based on a modification on disease levels and their matching scores defined by Couture (1980) earlier (Table A-2B). The scale provided comparable and differential scores for the reactions of barley genotypes to *R. secalis*. However, it seems that the scale was ill-defined. In many circumstances related to intermediate-scalding lines, without approaching maximum level of disease in the lower canopy, a low to intermediate level of disease are observed in the middle and upper canopy levels. Practically, this will lead to elimination of lines with slow-scalding resistance. The limits/cut-off points for selecting/eliminating genotypes in this scale was set so that the ratings of ≤4, 5 to 6, and 7 or higher were indicatives of resistant, intermediate, and susceptible reactions in one work (Burnett and Helm, 1995; Turkington *et al.*, 1998), and using the same scale, average scores of <2.1, 2.1-3.0 and >3 were considered resistant, moderately resistant, and susceptible reactions, respectively, by another (Xue *et al.*, 1994).

Although, it is not statistically acceptable to use 1 instead of 0 to correspond to no disease as it causes distortion (Couture, 1980), 1-9 scale has been also applied in several studies. Davidson and Wolfe (1985) tested different numerical rating scales and came up with a modified scale of 1-9 where the ratings of 1-3, 4-6, and 7-9, considered for levels of useful resistance to the breeding program, susceptibility of most existing cultivars, and fully susceptible lines that should be discarded, respectively. The scale was reported to be readily applicable to rating large numbers of lines. In the resistance group, the score 3 showed a severe infection in lower leaves and no lesions on the 2 uppermost leaves that is an equivalent to the score of 5 in Couture's scale which meant higher susceptibility than that expected for a resistance class. Another scale was also developed from the 0-9 scoring scale introduced by Saari and Prescott (1975) and modified into 1-9 assessment scale for diseases of cereal crops (Stubbs *et al.*, 1986). This 1-9 scale has been used by some scald workers with some modifications (Singh *et al.*, 2003; Wallwork, 1995; Yitbarek *et al.*, 1998). However, since each class is defined arbitrarily, there is no agreement between each class in terms of its relative disease severity. For example, a score of 5 may represent 15% diseased leaf area (Dr. H. Wallwork, personal communication) whereas the same score was given to a plant with 40-50% leaf area covered with the disease (Yitbarek *et al.*, 1998). As shown in Table A-2C, using another 1-9 percentage scale, Dr. K. G. Briggs (1995-97) defined the score of 1 to 9 for plants showing 0, 0-10, 11-23, 24-36, 37-50, 51-64, 65-77, 78-90 and 91-100% leaf area scald (Turkington *et al.*, 1998). A plant with a score of 5-6 or higher in this scale may receive a score of 8-9 according to the 0-9 scales. In this scale, most scores represent susceptible reactions whereas little space has been left for intermediate and resistant classes.

In Denmark, a 0-10 scoring scale has been developed for both official variety testing as well as the farmers' union trials where a combination of both disease severity and incidence has been incorporated (Dr. M. Rasmussen, personal communication). It can be considered as a good differential scale for resistant genotypes, however, it does not have adequate number of classes for intermediate scores. In this scale scores of 6, 7, 8, 9 and 10 correspond to 10, 25, 50, 75 and 100 percent leaf area diseased where it seems unnecessary to allocate separate scores for cases with over 50% leaf area diseased. Additionally, the differences between successive scores of 1, 2, 3, 4 and 5 which represent 0.01, 0.1, 0.5, 1.0 and 5.0, respectively, are negligible. In such a scale, the leaves in the upper canopy are considered almost doubly important than leaves in lower levels e.g. a plant with 50% infection on lower leaves and no symptoms on the upper canopy would be classified as a genotype with 25% of infection (Dr. S. Salamati, personal communication). Another 0-10 scoring scale was used to incorporate various factors such as lesion size, percentage of leaf area (severity) and plants (incidence), infected and size of scald foci in individual plots of eight rows, 3.5 m long (McDonald *et* *al.*, 1988). The scale is too detailed to follow and may not be usable for screening thousands of lines in scald nurseries.

Visual assessment of the percentage leaf area scalded (%LAS) has been regarded by many workers as useful means of evaluation of the disease severity at both seedling and adult plant stages (Jenkyn *et al.*, 1989; Khan *et al.*, 1984; Xue and Burnett, 1995; Yitbarek *et al.*, 1998). In the percentage scale, the upper and lower limits are clearly defined and the scale can be arbitrarily divided to sub-classes (James, 1971). In practice, levels of 100% infection may never be encountered (James, 1971), but the total chlorotic and necrotic area associated with the disease happens to reach 100%. The scores from the percentage data can be integrated over the season to measure the area under disease progress curve (AUDPC) and infection rate, which are indications of the amount and rate of disease development over the period of epidemic (Brown *et al.*, 1996; Habgood, 1975). A 1 (susceptible) to 9 (resistant) scoring system adapted from that used by the British Society of Plant Breeders was used to provide scores convertible to percentage infection (Newton *et al.*, 1997).

Standard area diagrams have also been incorporated into scald evaluation systems to increase the degree of accuracy desired for disease assessment (James, 1971). The scald standard area assessment key introduced by James (1971) has been used to either directly estimate %LAS (May and Harper, 1989) or indirectly score a plant in different ways (Xue and Burnett, 1995). James's (1971) assessment key for *R. secalis* only provides the rater with how to imaginary split a leaf to 10 equal segments of 10% and how to estimate an area covered with 1, 2 and 5 %. The assessor still needs to put in time to add up the total area covered with symptoms. Practically as in the scale of James, the process of rating would be faster/easier if the actual levels of disease on the leaves could be compared with illustrated leaves with a range of different diseased areas e.g. 1, 2, 5, 10, 15, 20, ..., 50% etc. (Kranz, 1988). Besides there is little similarity between what the scald lesions look like and what has been depicted in the scale. Hence, a new worker may require becoming familiar with symptoms of scald before undertaking the actual study. Furthermore, the scale has not been designed to integrate information about disease incidence and can not provide raters with simple one-digit scores to differentiate between the levels of reaction of seedlings and adult plants to *R. secalis*.

Using a scale of 0 – 7, 66 Canadian barley cultivars and breeding lines were studied for their reactions to the scald disease at both seedling and adult plant stages, where 0 = 0%, 1 = 1-5%, 2 = 6-10%,3 = 11-20%,4 = 21-30%,5 = 31-50%,6 = 51-75% and 7 = 76-100% (Xue *et al.*, 1995). The scale was a modification of another 0-11 scoring scale (Horsfall and Cowling, 1978), which contains 12 classes (Horsfall and Barratt, 1945). The number of classes in each rating scale may influence the accuracy of the estimate if the frequency distribution is skewed (Kranz, 1988). The number of disease classes representing intermediate and susceptible reactions in the 0-7 scale of Xue *et al.* (1995), did not look sufficient, whereas the 0-11 scale of Horsfall and Barratt (1945) had many upper classes which were unnecessary. In contrast, In the 0-11 scale, there were not an appropriate number of classes in the range near zero where most of resistance classes are assigned (Berger, 1981).

Canopy levels and growth stages in relation to adult plant assessment

For epidemiological and crop loss studies, disease severity has to be estimated on plant parts of the same age and canopy level (Teng and James, 2002). In barley, yield losses are known to correlate significantly with the disease severity of the two upper most leaves. Hence, there is a tendency to use the uppermost leaves of plant for disease evaluation (James, 1971). However, by assessing only the 2-3 uppermost leaves, one may not derive the best differential information possible from the evaluation. Also, epidemiologically, the importance of other leaves at the lower canopy levels cannot be ignored (Davis and Fitt, 1992).

Similarly, with respect to the importance of post-anthesis stage in several cereal diseases, it has been recommended to evaluate disease intensities between the medium milk (ZGS 75) and the soft dough (ZGS 85) stages of grains development (Couture, 1980). However, ratings in the early and intermediate stages of epidemics could also provide useful information about the impact of primary inoculum and its progress.

Table A-1. Scales which have been so far used for scoring barley scald at the seedling stage.

	Score					
Scale (#)	0	1	2	3	4	5
0-3 (1)	NS	SL* On tips or margins of leaves	LL** If marginal, extended along the blade	wilted leaves No evidence of discrete lesions		
0-3 (4)	NS	VSL (up to 3 mm)* Along the leaf margins, or round to oval	LL** Either at leaf margins, or in the centre of leaf	LCL or TCL Usually involving the leaf tip		
0-3 (6)	NS	SL * Usually on the leaf edge LAD < 10%	LL** LAD 10-40 %	LL ≥ 40% LAD		
0-4 (2)	NS	SL * At the tip/margin/base of leaf blade	Narrow band of lesions Extending down the length of the leaf blade	Broad well developed lesions ** Covering large areas across the leaf blade	Wilted leaves No evidence of discrete lesions	
0-4 (3)	NS	VSL Confined to leaf margins	SL* Not confined to leaf margins	LL** Involving the majority of the leaf area	TCL No distinct lesions	
0-5 (5)	NS	One or few small lesions often with a dark margin LAD < 5	Somewhat LL Irregular greyish-green area without dark margins * LAD < 10	Relatively LL with more often type 2 characteristics LAd 10-40	PCL Typically greyish-green areas with dark margin LAD 40-80	TCL with symptoms as the type 4 ** LAD 80-100

‡ (1) Schein (1958); (2) Ali and Boyd (1973); (3) Jackson and Webster (1976); (4) Tekauz (1991); (5) Jörgensen (1992), and (6) Jörgensen and Smedegaard-Petersen (1995).

* The level at/under which plants are classified as resistant.

** The level at/above whish plants are considered susceptible.

Abbreviations: NS = no symptoms; L = lesions; S =small; VS = very small; LL= large lesion; LCL= large coalescing lesions; TCL = total collapse of leaf; LAD= % lamina/leaf area diseased; PCL= partial collapse of leaf tissues.

Table A-2. Scales which have been so far used for scoring scald of barley caused by *Rhynchosporium secalis*. (A) University of Alberta scoring scale, (B) 0-9 assessment scale based on disease intensity at various leaf levels in cereals, and (C) A modified scale of Couture (1980).

A

	Score*									
Leaf Levels**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Upper	0	0	0	0	0	0	5	10-25	25-50	50
Middle	0	0	0	0	5-10	10-25	25	50	50	50
Lower	0	1	5	10	25-50	50	50	50	50	50

* In Couture's (1980) or its various modifications, disease intensities of 0, 1, 5, 10, 25, and 50% are described as isolated, scattered, light, moderate, and severe, respectively (Couture, 1980).

B

	Score*									
Canopy levels**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Upper	0	0	0	0	0	1	5	10-25	25-50	>50
Middle	0	0	0	1	5-10	10-25	25-50	>50	>50	>50
Lower	0	1	5	10	10-25	25-50	>50	>50	>50	>50

* Burnett and Helm's (1995) modified scoring system of Couture (1980) (Burnett and Helm's, 1995).

C

	Score*								
Canopy levels**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
% LAS	0	1-10	11-23	24-36	37-50	51-64	65-77	78-90	91-100

* University of Alberta 1-9 scoring scale (1995-1997) based on the percentage of leaf area scalded (% LAS) developed by Dr. K. G. Briggs (Turkington *et al.*, 1985).

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Appendix II: Supplementary tables for Chapter 3

Table A-3. Significance of pairwise least square means test for the final severity (FS) based on Tukey's method and the combined analysis of the western Canadian barley lines/cultivars trials carried out in Canada and Mexico.

† Ent.: Entry where according to Table 3-3, 1-49 represent cvs. CDC Silky to Brier(D), respectively.

* and ** significant at P< 0.05 and 0.01, respectively.

Ent†	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
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Canada

Mexico

Table A-4. Significance of pairwise least square means test for the terminal severity (TS) based on Tukey's method and the combined analysis of the western Canadian barley lines/cultivars trials carried out in Canada and Mexico.

† Ent.: Entry where according to Table 3-3, 1-49 represent cvs. CDC Silky to Brier(D), respectively.

* and ** significant at P< 0.05 and 0.01, respectively.

Ent¹	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
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Canada

Mexico

Table A-5. Significance of pairwise least square means test for the Standardized area under disease progress curve of the % severity (AS) and % incidence (AI) based on Tukey's method and the combined analysis of the western Canadian barley lines/cultivars trials carried out in Canada.

† Ent.: Entry where according to Table 3-3, 1-49 represent cvs. CDC Silky to Brier(D), respectively.

* and ** significant at P< 0.05 and 0.01, respectively.

Ent†	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
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AS

AI

Table A-6. Significance of pairwise least square means test for the apparent infection rat of the % severity (rS) and % incidence (rI) based on Tukey's method and the combined analysis of the western Canadian barley lines/cultivars trials carried out in Canada.

† Ent.: Entry where according to Table 3-3, 1-49 represent cvs. CDC Silky to Brier(D), respectively.

* and ** significant at P< 0.05 and 0.01, respectively.

Ent'	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
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